THE DEVELOPMENT AND CYTOLOGY OF RHODOCHYTRIUM[1]

ROBERT F. GRIGGS

(WITH PLATES XI–XVI)

To the student of phylogeny, whether he be taxonomist, or morphologist, no organisms are as interesting as those which appear to occupy positions intermediate between the larger groups and help to fill the gaps in our evolutionary system. Such a form is *Rhodochytrium*, for it seems to occupy a transitional position between the protococcoid algae and some of the chytridiaceous fungi. It was described by its discoverer as an alga, but it has no chlorophyll and is strictly parasitic in its mode of life, being limited, moreover, to definite host species. Although entirely incapable of photosynthesis, it develops abundant starch. But the starch grains are apparently built up directly in the cytoplasm, for neither plastids nor pyrenoids have been found. This paradoxical combination of characters aroused in the writer a desire to investigate the details of its structure and to compare its cytology with that of *Synchytrium*, which has proved so peculiar.

As is well known, *Rhodochytrium* has been found only in three widely separated regions. LAGERHEIM observed it in many places in Ecuador on *Spilanthis* sp., and his material has been distributed in WITTROCK and NORDSTEDT'S *Algae Exsiccatae* as no. 1096. BARTHOLOMEW discovered it on *Asclepias pumila* about 20 miles from Stockton, Kansas, and distributed it as *Fungi Columbiani* no. 2166 (forma *asclepiadis* Farlow); and finally STEVENS and HALL[2] have found it on *Ambrosia artemisiifolia* in many places in North Carolina, as reported by ATKINSON (3). BARTHOLOMEW has informed me that the plant is rare in Kansas and known to him from only one locality, but both in Ecuador and North Carolina

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[2] MR. HALL has also found it at Clemson, South Carolina.

it is widely distributed and common. It is in each case, however, except possibly in Kansas, closely limited to the particular host on which it was reported.

The parasite attacks all the aerial parts of its host, but, like certain species of *Synchytrium*, it is largely confined to the tissues immediately adjoining the vascular bundles. To the naked eye each parasite appears as a small bright red spot buried in the tissue of the host. When a piece of the infected tissue is examined under the microscope, it may be seen that the parasites are of two sorts, resting spores and zoosporangia. The resting spores are somewhat deeply buried in the tissue of the host, but their superficial origin may be demonstrated by the persistence of the original germ tube with its external button, *Cystenhäut* (fig. 4), through which the parasite penetrated the host. The zoosporangia (fig. 28) are irregularly turbinate or retort-shaped bodies with wide flaring necks, through which their contents are emptied at maturity as biciliate zoospores which spread the infection during the growing season. From the basal portions of both sorts of cysts numerous rhizoids are given off, which penetrate the vascular bundles of the host, especially their phloem elements, and gather nutrient for the parasite.

In carrying out the investigation I have been aided to an unusual degree by my friends. I desire to extend my thanks and acknowledgments to Messrs. F. L. STEVENS and J. G. HALL of the North Carolina Experiment Station for the material, especially to the latter gentleman, who has put himself to no little inconvenience in killing material at all hours of the night as well as in seasons of the year when it was difficult to secure; to Professor GEORGE F. ATKINSON, who himself planned to make detailed studies upon the plant, for the generous way in which he encouraged me to proceed with the present investigation; and to Professors ROLAND THAXTER and W. G. FARLOW for the courtesies of their laboratory and for much valuable criticism and advice.

The material was killed at Raleigh in chromacetic acid and shipped to Columbus in the killing fluid, after which it was dehydrated and imbedded in paraffine in the usual way. The safranin-violet combination proved most satisfactory as a stain. Iron

haematoxylon, for some reason, was hard to handle with this material.

Observations on living material

Through the kindness of Mr. HALL, supplies of infected ragweed were sent to me at frequent intervals. By this means it was possible to determine the approximate sequence of events through the year. It would be interesting to compare the seasonal cycle of the parasites in North Carolina and in Ecuador, but LAGERHEIM has left us no data concerning the seasonal history of his plant. In regard to the characters of the living cysts and the behavior of the zoospores, I cannot add in any important particular to LAGERHEIM'S account, though my observations confirm his at almost every point.

According to my observation, the parasite does not appear at Raleigh until rather late in the season. Seedling ragweeds, gathered among the stubble containing the old resting spores on April 20 and May 20, showed no infection on arrival in Columbus, and did not subsequently develop any when grown in the greenhouse. Young plants gathered May 31, however, showed a few parasites. At first nearly all of the cysts become zoosporangia, but before June has passed, resting spores begin to appear in numbers, the zoosporangia become gradually scarcer and scarcer, until finally, about August 1, practically the only cysts found are the quiescent resting spores which undergo no further change until the following spring. These are even more conspicuous than the zoosporangia, but for any observations, either biological or cytological, material must be gathered while zoosporangia are still abundant, that is to say before the middle of July, preferably during the latter half of June.

With a little care the two sorts of cysts can be distinguished in the living state under a hand lens. The resting spores are more regular in shape and more deeply buried than the zoosporangia, and they are usually more deeply pigmented, since their protoplasm is more compact and less vacuolate.

The first infections observed were mostly on early leaves, which soon wither and drop off in the natural development of the plant,

whether parasitized or not. But before these leaves wither, the parasites they contain ripen and discharge their zoospores, which carry the infection to the younger parts nearer the growing point of the host. In this manner infection is carried to successively higher and higher levels of the growing plant, until the host is often red with parasites.

LAGERHEIM believed that the cysts arose not only by direct infection but also by proliferation from the mycelium of old ones. If this occurred, a single infection might, by repeated proliferation, infect every part of the host plant. But in the form on *Ambrosia* no indication of such proliferation was found. Nowhere among the rhizoids were any indications observed of the formation of new growing points or other signs of proliferation. During the whole of the growth period the parasite is strictly unicellular, with a single nucleus in the body of the cyst, and when nuclear division begins preparatory to sporulation, the nuclei do not wander into the rhizoids. In sectioned material only a small proportion of the parasites are so oriented that a single section passes centrally through the whole cyst. But in no case, where the series was complete, was there any difficulty in finding the external opening of any zoosporangium, whereas if proliferation had been occurring, numerous partially formed cysts which had not yet grown out to the epidermis should have been encountered. In those parasites which become resting spores the independence of the cysts cannot be demonstrated by finding their external openings, because, on account of the narrowness of their necks, only a small proportion of them can be followed to the exterior. If proliferation occurred, the new cysts could become nucleated only by migration of nuclei through the rhizoids. But not only do the nuclei of the resting spores remain undivided, but they have not been seen to wander from their central position in the middle of the cyst, and they are so large that it is difficult to imagine them squeezing through the rhizoids.

Although the parasites are so abundant as almost to cover the host plant, and the rhizoids destroy the cells which they penetrate, the vigor of the plant is little impaired. But when infected ragweeds are transplanted, it is difficult to prevent the parasitized

leaves from withering and dying, and reinfections on healthy portions of the plant are difficult to secure.

The most convenient way to obtain the zoospores is to tease to pieces fragments of tissue containing the cysts, liberating the zoospores by rupturing the sporangia. It is difficult to observe the normal exit of the zoospores on account of their minuteness as compared with the massive tissues from which they emerge. But with patience one can study the discharge. LAGERHEIM states that the plug of the zoosporangium is dissolved before the escape of the spores. In only one case was I able to observe the discharge under satisfactory conditions, and then I saw neither the fate of the plug nor the very beginning of the discharge. The whole mass of zoospores appeared to expand as swarming began, and those nearest the opening were forced out in a solid stream by the pressure of those below them. In the case observed they continued to escape at the rate of about 150 per minute for 10 minutes (that is, approximately 1500 spores). The last ones from the rhizoidal end of the sporangium were not at first so well formed as the others, and did not escape with them, but after an interval of 5 minutes began to swarm violently inside the sporangium and some of them escaped one by one. Not all of them were able to find the opening, however, and those which failed became quiescent after about 15 minutes.

Along with the ripe zoosporangia many immature ones, of course, are torn open in teasing apart the material for mounting. Such of these as have advanced far, though not yet mature, are apparently able to form zoospores under the stimulus of rupture. When first discharged the contents of these cysts undergo euglenoid contortions, but in a few minutes become ciliated and break up into spores. Such zoospores, however, are very irregular in size, and abnormal forms compounded of several individual spores are common. Among these are some which might easily be confused with conjugating gametes, being associated in pairs side by side. More commonly such double zoospores are joined at their posterior ends, forming much elongated bodies, pigmented and ciliate at both ends. Frequently a third member is attached to the middle of such a couple, forming a projection at right angles. Others are

large multiple bodies with four or more pigmented areas and many cilia. Such abnormal spores, of course, have very erratic and peculiar movements. Their period of activity is short, few continuing to swim actively for more than half an hour. LAGERHEIM observed these same abnormal spores, and inferred from them that segmentation was successive rather than simultaneous, but, as will be seen, this is not the case.

The zoospores are transparent, except at the anterior end, which is occupied by a mass of pigment. After they come to rest the nucleus can be seen distinctly as a clear central vacuole. In the posterior portions are numerous granules, usually including some starch grains. When moving most actively, the zoospores are oblong rather than pyriform, as figured by LAGERHEIM. Indeed, they appear to be narrower in the region of the nucleus than in the anterior pigmented end. But this is believed to be due to an optical illusion, the more conspicuous region irresistibly appearing larger. It is of course not susceptible of careful observation, since the shape changes at once when they come to rest.

If plentifully supplied with fresh water, the zoospores continue to swim about actively for several hours. In numerous instances they were watched for half a day at a time, and in one case the last one on the slide did not perish until 8 hours after liberation. In preparations supplied with abundant water conjugation occurs but seldom, according to my experience. But when the water has evaporated to a considerable extent, all begin to conjugate at once. When more water was added, those pairs in which fusion had not proceeded too far dissociated rapidly and swam about singly as before. From this it was suspected that conjugation might be due to the increasing osmotic pressure of the medium consequent upon evaporation. On this supposition a few crystals of sugar were added to a similar preparation, making the concentration very much higher than on evaporation, but this had no apparent effect on the zoospores. It was therefore concluded that conjugation was induced by an insufficiency in the quantity of fluid present, and this conclusion seemed to be confirmed when on placing two portions of a culture of zoospores, one in very scanty and the other in abundant water, the first quickly conjugated while the second

did not. The process of conjugation is not different from that common in various algae. Two zoospores of approximately equal size approach (fig. 38) and lie alongside each other (figs. 39, 40); the plasma membranes separating them disappear, and within a few minutes the nuclei, which may be seen as clear central bodies, have fused into one (fig. 41). The two pairs of cilia remain distinct, and in the cases observed by me there persists a slight groove in the anterior portion of the zygote indicating the line of fusion.

LAGERHEIM reports that both conjugated and unconjugated zoospores are able to infect the host. My own observations on this point gave no results. Repeated efforts were made to observe the process of infection, but for the most part the spores swam indifferently about the pieces of fresh ragweed which were placed on the slide with them. In some cases, indeed, the spores, both conjugated and single, settled down on such pieces of the host and became fastened to them with one or more cilia, but in no case did penetration occur.

My attempts at reinfection on the living plant were similarly unsatisfactory. Out of numerous attempts, only three successful infections were secured. In these cases the development of the young parasites was very rapid, but as the successful experiments were my first attempts in that direction, and as all efforts to repeat them failed, I do not feel warranted in reporting them in detail.

One of the interesting questions which the failure of the infection experiments left unsolved is how the character of the young cysts is determined, that is, whether they are to develop into resting spores or into zoosporangia. Although this is connected with the seasonal cycle in North Carolina, there is no indication in LAGERHEIM'S account that such is the case in Ecuador. By analogy with other forms, one might suspect that the zoosporangia spring from unconjugated zoospores and the resting spores from zoozygospores. But there is no definite alternation of generations, as in some such forms. In any case, the character of the cyst appears to be determined immediately on infection. As may be seen from the figures, the methods of penetration and growth are different from the very beginning, so that in the very youngest cysts there is no question whatever which are zoosporangia and which are resting spores.

In very few cases was there any ambiguity in this respect, although several malformed zoosporangia were seen. In one of these a heavy wall had formed across the neck, leaving only a small pore between the neck and the body of the cyst. Several very narrow-necked zoosporangia were also observed, but though these resembled the resting cysts in shape, they were apparently otherwise normal.

MICROCHEMICAL REACTIONS.—The two outer walls of the resting spores are cellulose, as reported by LAGERHEIM, who used chlor-zinc-iodide as a test reagent. With iodine and sulphuric acid also they give the cellulose reaction, but were not in my tests as deep a blue as the cotton fibers which were used as a check. But the endospore is different in character and was unaffected by any of the reagents or stains employed.

LAGERHEIM suspected that there might be chlorophyll in some stage of the life cycle, though he was not able to detect it. The plant has more or less red pigment at all stages, but none of my observations gave any ground for supposing chlorophyll to be present.

The red pigment, as reported by LAGERHEIM, is haematochrome or some closely related lipochrome. It is colored green with iodine in potassium iodide, blue with sulphuric and nitric acids, fading away after treatment with the latter. Tests with red individuals of *Sphaerella* under the same cover-glass with *Rhodochytrium* gave somewhat contradictory results, but showed some differences between the pigments of the two. The haematochrome of *Sphaerella* was not dissolved by carbon disulphide, which is a solvent for the allied pigment carotin, even after prolonged treatment, but the pigment of *Rhodochytrium* was easily dissolved under the same conditions. The haematochrome reacted to a weak solution of iodine such as is used for testing starch, but the pigment of *Rhodochytrium* remained unchanged until a strong solution of iodine was applied, when the characteristic reaction appeared. With sulphuric acid also *Sphaerella* reacted instantly, but drops of the red oil of *Rhodochytrium* remained unchanged for several minutes and slowly turned blue. While still inclosed in the unbroken spore, the pigment is very resistant to almost all reagents. This was first noticed on fixing with chromacetic acid, which fades out

almost everything put into it. Nor did the color fade during the prolonged soaking in alcohol and hot chloroform incident to imbedding in paraffine. But when after sectioning it was treated with turpentine, it quickly dissolved. Although easily soluble in carbon disulphide after the spores are broken open, as stated above, it resists that reagent indefinitely (three months) when bits of the host plant containing the spores are treated with it. It was likewise unaffected by three months' treatment with xylol, benzene, chloroform, absolute alcohol, ether, and turpentine. It was also undimmed in brilliance after 6 days' maceration in 10 per cent hydrofluoric acid.

One of the most conspicuous features of the material was the great difference in certain respects between that collected in 1908 and that in 1910. In the former the zoospores (figs. 32–34), and for the most part the zoosporangia also, after the first division (fig. 27) were entirely destitute of starch, their cytoplasm being clear and finely granular. But in the latter the zoosporangia (figs. 15, 27) and almost all of the zoospores as well (fig. 35) were abundantly supplied with starch, which on account of its refractive and staining properties greatly interfered with the observation of nuclear phenomena. The condition of the zoospores was of course reflected in the young cysts, which in 1908 had at first clear granular cytoplasm without a sign of starch grains or any other structures (figs. 2, 3, 14), while in 1910 the cytoplasm was packed with small starch grains from the first (figs. 1, 11, 12). There were also some differences in the nuclear behavior in the two cases. Those figures which are interpreted as amitosis are almost entirely confined to the 1908 material. The plugs of the zoosporangia are also very different in the material of the two years as described below (p. 136). Moreover, there is reason to believe that similar variations occur in the Ecuadorean form, because there are discrepancies between LAGERHEIM'S account and that portion of his material which I have examined, which would be inexplicable to me if I knew only the 1908 material of the form on *Ambrosia*. These differences serve to emphasize the caution we must use in interpreting cytological results. They can hardly fail to suggest that some of the numerous instances where one investigator does not

find what another has reported in a given species, may be due to variations in the conditions of the environment at the time of collection, the effects of which are almost entirely unknown at the present time. From the very character of the work, such errors are peculiarly liable in cytological investigations, for it is manifestly impossible within reasonable limits of time to examine thoroughly material taken under various conditions of growth over a series of years.

The question of species

On account of the great distances separating the three known habitats of *Rhodochytrium* and the diversity of its hosts, one is led to suspect that there are three species rather than one. With the idea of separating them if possible, a study was made of LAGERHEIM'S and of BARTHOLOMEW'S collections. Previous comparison of the North Carolina material with LAGERHEIM'S description had disclosed some minor differences, but these disappeared on examination of the plant itself. In the form on *Asclepias*, likewise, I am entirely unable to detect any constant or significant differences. The various figures presented herewith show how difficult it is to find characters in *Rhodochytrium*. In size and shape there is every possible variation, and there is a total absence of such peculiarities as markings on the spores, etc., which in many groups supply useful specific characters.

It was thought for a time that the shape and size of the plugs which close the mouths of the zoosporangia were different in the three forms. LAGERHEIM describes the original *R. spilanthidis* as having a bell-shaped plug (cf. fig. 21) which did not develop until the sporangium had reached a considerable size. In the form on *Ambrosia* the plug is generally 25–35 μ long, solid, and develops early (fig. 14). The form on *Asclepias* has a similar plug, but it is usually larger, reaching a length of 60 μ (fig. 16). The condition of all the plugs in the 1908 material was fairly constant, but the 1910 material showed such variation that it became evident that the characters of the plug were worthless. Its size varies with that of the sporangium. In large sporangia on the stem it sometimes reaches 50 μ in length, and in small ones on the leaves it is

sometimes as small as 12 μ. Moreover, it is sometimes very tardy in its development. The variation in shape is likewise great (figs. 15, 17, 18, 20, 21, 24). LAGERHEIM'S material shows for the most part the same sort of solid plugs. The form shown in fig. 19 was observed but twice, while bell-shaped plugs such as he figures were entirely absent from that portion of his material which I examined. It seems safe to assume that the apparent discrepancy is to be explained by the same sort of variation as that just noted in the form on *Ambrosia*.

There seems, therefore, to be no course open but to conclude that there is no morphological basis for separating the three forms. There is, on the other hand, reason to expect that they would be found to be physiologically differentiated in respect to their hosts if a series of experiments in cross infection were undertaken with one or all of the forms. Until the experimenter acquires more skill, however, than is possessed by the writer in transferring the infection from plant to plant, the expected negative results of cross-infection would prove nothing.

The range of the plant seems to call for some comment, but the data are hardly sufficient to decide whether the three known localities represent points in a single extensive range, or whether they are isolated stations. If they represent the continuous range of a single species, the limitation to such unrelated hosts raises some considerable difficulties concerning their distribution. Two of the hosts, *Spilanthes lundi* and *Asclepias pumila*, are somewhat localized species, and their range in neither case extends to either of the other stations; but *Ambrosia artemisiifolia* is widespread, and occurs both in Kansas and throughout South America. If the three forms are not physiologically distinct, therefore, cross-infection should occur naturally in Ecuador.

It seems, therefore, that the answer to the question of the number of species of *Rhodochytrium* will depend on the point of view of the student. He to whom geographical and physiological isolation are criteria of species may well conclude that there are three species, while he who demands morphological characters by which to distinguish species will decide that there is but one. Each of these points of view has its advantages, and it is not for

the writer to determine which shall be adopted by his readers. In some groups, as in the bacteria, species are perforce determined almost exclusively by physiological characters, while in other groups, as in the seed plants, morphology alone determines the matter. In the parasitic fungi various infection experiments have shown that numerous species which occur on several hosts may be composed of physiological races, each confined to its particular host. Such a treatment seems to the writer an entirely satisfactory manner of expressing the facts, and he does not see that there would be any gain in considering the forms specifically distinct.

The development of the resting spores

Although the resting spores do not appear in numbers until several generations of zoosporangia have matured and discharged, it will be more convenient to describe them before the more complex development of the zoosporangia is taken up. The very youngest resting spores seen measure about 70 μ in length (fig. 1). They consist of an elongated germ tube with an external button marking the position and size of the zoospore from which they originated. The distal end has already begun to enlarge, but the nuclei (5 μ) are not much larger than those of the zoospores. The germ tubes do not seek out the stomata even when close beside them (fig. 11), but force their way between the epidermal cells at any point. After penetrating a variable distance, usually until a vascular bundle has been reached, the tube begins to swell up and gradually it acquires a globular form. The swelling out of the cyst is very much more rapid than the growth of the protoplast, which in consequence becomes highly vacuolate (fig. 2), like an old cell far back from the growing point in an ordinary plant. There is an attenuate peripheral layer of cytoplasm connected by radial strands with the central body surrounding the nucleus, which likewise has grown but little. At the very beginning of the enlargement of the basal portion, the protoplast withdraws from the narrow neck of the germ tube, which is later cut off by a wall.

Even when full sized, the parasite distorts the tissues of the host but very little. Most of the cells which lie adjacent to it appear as though cut off to make room for its growth rather than crowded

aside by gradual pressure (fig. 15). Generally the walls of these cells can be readily distinguished from that of the cyst, though they may be closely appressed to it. Such walls usually correspond approximately in length with the adjacent part of the parasite. This indicates, especially in those cells that have been much reduced in size, that they have shrunken considerably, for the original wall would have been much crumpled if merely pushed back by the expanding parasite. They often lose their sharp outlines and appear to be undergoing digestion.

The supply of nutriment which makes possible the growth of the parasite is drawn from an extensive system of haustorial rhizoids, which are put out from the basal portion of the young parasite even before the germ tube begins to swell out into the spherical cyst. They continue to increase and to extend their ramifications until the cyst reaches its full size and begins to ripen, finally extending considerable distances along the vascular bundles. But notwithstanding the wide extension of these elements and their filamentous form, they can hardly be compared with the hyphae of a true fungus. They are by no means to be looked upon as the vegetative portion of the plant from which the fruiting bodies take their origin, but merely as rhizoidal outgrowths from the main body of the parasite. When old they develop thick walls, especially in the portions close to the cyst. But at the extremities, where most of the absorption may be supposed to occur, the wall is exceedingly delicate or invisible. Although they sometimes work their way between the disorganizing cells, their course is for the most part within the cells which they invade (fig. 5), and their shape is often largely determined by the boundaries of these cells. Both LAGERHEIM and ATKINSON speak only of those haustorial branches which become attached to the vessels of the system. But the great mass of the rhizoidal system is located in the phloem (figs. 4, 5, 15, 22), and it is the cells of the phloem which are most injured, finally breaking down completely, while the xylem is but little injured. It must also be obvious that the vessels could not furnish the supply of organic food necessary to nourish the parasite. There is no doubt, however, but that some of the ultimate branches of the haustoria do come into close relation

with the vessels, exactly as described by LAGERHEIM and ATKINSON (fig. 5), and probably draw water from them. These terminal haustoria (fig. 6) are closely appressed to the thin places between the spiral thickenings of the vessels, but appear not to penetrate them as in the phloem.

With the development of the rhizoids the protoplasmic contents of the cysts become more abundant and denser. The nucleus increases in size and undergoes a metamorphosis like that of the zoosporangium described below. Starch grains, if not already present, appear and become large and abundant, until they pack the cyst so full that its cytoplasmic contents proper may become almost invisible. In this process all vacuoles disappear and apparently all surplus water is eliminated. Even the aqueous karyolymph partially disappears, causing the nucleus to collapse (figs. 7, 8). In this condition the nucleus differs so far from ordinary healthy nuclei that it is difficult to believe that this change is not pathological. But it seems to be a universal and perfectly normal phenomenon. On the beginning of germination in the spring, the nuclei again become turgid, though they are apparently smaller than before shriveling up.

When the vegetative activity of the parasite is ended, as indicated by the shriveling of the nucleus and the withdrawal of all of the starch from the rhizoids into the spore, a second cellulose wall is laid down on the inside of the spore (fig. 7) and sometimes in the proximal ends of the rhizoids as well (fig. 5). But either at the time of deposition of the second layer of the spore wall or soon afterward, the rhizoids are cut off from the spore first by a plasma membrane and later by a definite wall. This is soon followed by the disorganization of the contents of the rhizoids. The second wall of the spore is quickly followed by the formation of a third (fig. 8), a thick, non-cellulose endospore, which completes the preparation of the spore for its period of rest.

The starch grains

One of the most interesting things about *Rhodochytrium* is the fact that though it is a parasite and has completely lost its chlorophyll, it forms starch in considerable quantities. The source of

this starch is of course the photosynthetic activity of the host, but it is hardly necessary to state that the starch grains of *Rhodochytrium* are quite different in form from those of the adjacent host cells.

As would be expected, starch is most abundant and best developed in the mature resting spores, in which it forms the bulk of the reserve food, but it may be present at any stage in the life cycle. In the zoosporangia it is nearly always present toward the end of the vegetative period, but there is a decided tendency to consume it during the period of nuclear division. A marked difference was noted in respect to starch content between zoosporangia gathered in 1910 and those gathered in 1908. In the former both zoospores and very young sporangia contain numerous starch grains, but in the latter starch appears tardily and almost always disappears before segmentation, leaving the cytoplasm clear and granular, without inclusions of any sort.

The grains seldom exceed 10 μ in diameter and are commonly somewhat smaller. They are usually spherical or somewhat elongated, but very long or double grains are not rare (fig. 9). The larger grains when mounted in balsam frequently show conspicuous cracks at the hilum, as is not unusual in starch grains generally. No definite alternating concentric layers of different refractive indices such as characterize many starch grains could be made out, but in certain grains faint concentric striae appeared to be present. When subjected to the action of strong chromic acid, they show during dissolution the radial structure characteristic of starch grains in general.

STARCH GRAINS UNDER POLARIZED LIGHT.—In the dark field obtained by crossing Nicol prisms, the starch grains show the usual luminous body crossed by dark bars in the two planes of polarization (fig. 10). But there is considerable variation in the behavior of different grains, both in those of the same cyst and in different cysts taken as a whole. Almost all conditions, however, may usually be found in a single cyst. Many of the spherical grains show no change other than the revolution of the crosses when the prisms are rotated, demonstrating in these grains a perfectly symmetrical structure, with the hilum occupying a point in the

center. In elongated grains the crosses resemble those in leguminous starch, namely, a pair of **Y**s arranged bottom to bottom, indicating an elongated hilum. And in double grains, which are not infrequent, the stems of the **Y**s are sometimes divided, so that the very center of the grain appears bright. Such grains are of course unsymmetrical, and show the characteristic crosses only when the planes of polarization form the proper angle with the axes of the grain. There are also great differences in the brilliance of the grains; some are very beautiful objects, but others repolarize the light to such a slight extent that they are very faint and the dark crosses are difficult to see. Frequently, indeed, the grains become entirely black and vanish completely when the prisms are crossed. When this happened, I was inclined to suspect that I might have mistaken grains of some other substance for starch, but on running iodine under the cover the characteristic blue reaction promptly appeared to dispel all such doubts.

ABSENCE OF PLASTIDS.—It is unsafe to assert, perhaps, that there are no plastids in *Rhodochytrium*, but it is certain that methods which bring them out clearly in such objects as old potato tubers failed to reveal them in *Rhodochytrium*. So far as could be determined, the starch grains are formed directly in the cytoplasm without the intervention of plastids, pyrenoids, or other specialized protoplasmic bodies. There was only one feature which could be taken to give any indication of such bodies. Many of the grains do not stain uniformly throughout, but show a more deeply colored margin. This appearance is not confined to grains of any particular size, but is found from the smallest to the largest grains. Indeed, when present at all the border is usually wider and more conspicuous in the large grains than in the small. It occurs rather on certain slides or perhaps on certain pieces of material, being present in nearly all of the cysts of some slides while absent from others. The border appears to have the same crystalline structure as the rest of the grain, and seems definitely to be a part of it rather than a separate surrounding body. In no case did it present the granular appearance to be expected of a plastid. I have no satisfactory explanation to offer for this phenomenon, but I do not believe it is permissible to interpret it as a plastid.

Almost ideal conditions for observation of the process of starch formation are sometimes presented in very young zoosporangia (fig. 11), where the cysts are highly vacuolate, with delicate strands of cytoplasm stretched from side to side. In thin sections such strands are suspended across the cyst, with no adjacent objects to interfere with vision. Frequently these strands show all stages in starch formation (fig. 11*a*) from good sized grains down. The larger grains are clear cut, sharply outlined against the clear cytoplasm in which they are suspended. From such well-formed grains there is an unbroken series of smaller and smaller grains down to the limit of visibility. The very earliest stages appear as mere knots in the cytoplasm, while the definite characters of starch grains appear as soon as the body reaches a size large enough to be resolvable into an area rather than a point. At no stage was anything seen in association with the starch grains except morphologically undifferentiated cytoplasm. More often, of course, the grains are formed in large masses of cytoplasm where the opportunities of vision are not so good, but here also they appear to lie naked in the cytoplasm.

The classic examples of the formation of starch grains without differentiated plastids were described by STRASBURGER (**26,** pp. 155 ff.). He found that in the megaspores of *Marsilea* and in the medullary rays of *Pinus* the growing grain was invested by numerous microsomes, which he believed secreted the starch in a manner analogous to the formation of the cell wall by the granules of the spindle fibers at the close of mitosis. These microsomes were large enough to appear as definite granules under a comparatively slight magnification (450 diameters). In *Rhodochytrium*, however, no such microsomes could be made out under a magnification seven times as great.

It should be added also that in those stages where starch is absent the cytoplasm is smooth and granular, without inclusions of any sort. If perchance the writer had overlooked the plastids among the grains during starch formation, he would have expected to see them here, if present. If there are any plastids, therefore, they would appear to be formed *de novo* rather than carried over from generation to generation as permanent organs of the cell.

The development of the zoosporangia

As already stated, the zoosporangia are distinct from the resting spores from the very beginning. The youngest stages seen were approximately as large as the youngest resting cysts, namely, 60–80 μ in length. These future zoosporangia do not form external buttons, and the neck, even at the very first, is of comparatively large diameter (fig. 12). While still very young, the cyst begins to swell out from the initial tubular form, and soon assumes the roughly turbinate shape characteristic of the mature zoosporangium. But before the parasite begins to expand, it generally penetrates straight into the tissues until it has reached the vicinity of a vascular bundle. The final size of the cyst is roughly proportional to the length attained by the germ tube, but of course the relation is somewhat accidental, since it is the stronger bundles capable of supplying more abundant food which are the more deeply buried. In the leaves the distance is approximately 100 μ, while in the stems, where the vascular bundles are relatively deeply buried beneath the cortex, a length of 300 μ or more is frequently attained (fig. 14). It thus happens that size is no criterion of the age of a cyst, some uninucleate cysts being much larger than some which are far along in division, as shown by figs. 12 and 26, which are drawn to the same scale.

Sometimes, while still in the tubular condition and usually before full size has been reached, a characteristic plug is formed at the mouth of the zoosporangium. In all but the youngest stages this is the most convenient character for distinguishing the zoosporangia from the resting spores, since the latter never develop a plug. But the plug is subject to great variations in size, and in rare instances may never develop at all. The most typical form is a solid top-shaped mass which stains deeply and uniformly throughout (figs. 14, 15, etc.). Often it is a hollow, bell-shaped structure (fig. 21), as figured by LAGERHEIM (see above, p. 136). In some instances such bell-shaped plugs were found to be perforated so as to place the interior of the cyst in open communication with the outside. Some solid plugs were observed which stained lightly, except on the lateral edges (fig. 24), giving the appearance of bell-shaped plugs which had been later filled up. In many cases the

plug is secondarily surrounded by several concentric layers of material, evidently laid down at intervals. Such plugs show great variation in appearance (figs. 17–20), presumably on account of variations in the conditions of deposition.

As in the resting spore, the protoplast is at first highly vacuolate, consisting of a peripheral layer of cytoplasm connected with the central mass about the nucleus by radiating strands. As growth proceeds, the cytoplasm becomes more abundant in proportion to the vacuoles, but the zoosporangia always have larger vacuoles than the resting spores. Sporangia of different ages, however, vary considerably in this regard. The larger cysts usually have larger vacuoles than the smaller. In later stages there is always one large vacuole which occupies the upper half of the cyst, the protoplasmic contents, except for a thin peripheral layer, being confined to the basal portion, as shown in the figures. The numerous rhizoids which are put out from the base are like those of the resting spore.

The cysts reach full size before there is any indication of division. But when division commences, the binucleate, tetranucleate, and later stages follow each other in rapid succession (figs. 22–28), until a large but variable number of nuclei have been formed. Upon completion of the period of nuclear division, segmentation occurs and zoospores are produced. The coenocytic cysts are comparatively rare. Never, even in the most favorable material, do they approach in abundance the primary cysts or those in which segmentation is complete.

The shape of the cysts seems to be determined largely by accidental variations in the compactness of the tissues in which they lie. The penetrating germ tubes follow to a large extent the path of least resistance. This sometimes leads them to spread out in the tissues (fig. 2), and causes considerable irregularity in the form of the mature cyst.

In those cysts which have abundant starch, clear spaces, roughly corresponding in size and shape with the primary nuclei, persist for some time after division (figs. 22, 57). Similar appearances are found sometimes in the telophases of the later mitoses (fig. 66). These are not vacuoles, as might at first appear from contrast

with the starch-filled cytoplasm surrounding them, but are occupied by cytoplasm similar to that of the remainder of the cyst, except that it is free from starch. This condition endures for a variable period; it sometimes disappears during the binucleate stage (cf. fig. 23), and sometimes persists into the octinucleate stage (fig. 24).

The nuclei of the early stages of the coenocyte tend to remain in the central position originally occupied by the primary nucleus, but later scatter, finally becoming evenly distributed through the cytoplasm. The period at which they disperse varies, as would be expected. One case was found in which they were still closely bunched in the 16-nucleate stage (fig. 25), but they are usually dispersed a little before that time.

SEGMENTATION.—On account of certain apparently conflicting processes observed, the writer has not been able to satisfy himself altogether concerning the mechanism by which the coenocytic cyst is cut up into spores. The account here given is therefore somewhat tentative.

During the last mitoses in the sporangium, a change seems to come over the protoplasm of the coenocyte. Up to this time the nuclei have apparently lain freely in the common cytoplasm without any tendency to form separate cells. But during these mitoses the cytoplasm appears to contract around the spindles and to draw up closer to them, so as to leave vacuoles in the intermediate spaces (fig. 30). These vacuoles, surrounding, as they do, the separated masses, often resemble cleavage furrows cutting the coenocyte up into individual cells. The cytoplasmic edges of the segments do not present the sharp clean outlines seen in progressive cleavage, however, but appear more or less irregularly frayed, and frequently cytoplasmic strands cross the vacuoles and connect adjacent masses.

These connections would seem to put aside any interpretation of the process as due to cleavage furrows, but one cyst was observed in which the margins of the individual masses were clear and sharp, without any bridges across the furrows (fig. 29). This case was difficult to interpret otherwise than as progressive segmentation by cleavage furrows.

This cytoplasmic contraction appears to be a universal occurrence, having been seen in all of the numerous cysts of this age observed. Nevertheless, in the writer's judgment it is not to be interpreted as segmentation. That appears to be a distinct process of a different nature. Since the preliminary contraction occurs during mitosis, it gives rise not to uninucleate but to binucleate segments. No indication of a constriction separating the daughter nuclei was seen in the telophases observed (figs. 63–65). The steps connecting this condition with what I take to be true segmentation could not be made out, but it would seem probable that the contraction disappears after mitosis is complete and the protoplasm of the cyst again becomes a continuous coenocyte. It will be understood that a regressive change of this character would be difficult to demonstrate except in living material, which in *Rhodochytrium* is too thick and too deeply pigmented to permit the observation of details of this sort. If the zoospores were always the same size, or if segmentation always occurred after a given number of nuclear divisions, it might be possible to recognize those cysts which had passed through their last mitosis and were ready for the final segmentation, but both the size of the zoospores and the number formed in different sporangia vary to such an extent as to make it impossible to distinguish those sporangia which have completed the cycle of mitosis from those which have not.

But whether the cysts again become continuous coenocytes or not, there is another sort of cleavage, which I take to be true segmentation, that appears to delimit the spores without reference to the separations brought about during the preliminary contraction. This occurs by the precipitation of membranes around the protoplasmic units (fig. 31). Each nucleus with its quota of cytoplasm is cut off from the rest by a membrane which appears within the strands of cytoplasm after the fashion of free cell formation in the endosperm of a seed plant. The membranes of the protospores are very delicate, but the method of their formation seems to be clearly indicated in the preparations. If one observes a protospore which is not yet completely surrounded, the terminal portion of the advancing membrane will appear simply as a heavy

strand of cytoplasm (fig. 31, *a*). The spores seem to round off soon after their membranes are laid down, presenting as they do so somewhat the appearance of bodies being divided by advancing cleavage furrows. Observation of the terminal portions of the apparent furrows shows, however, that they merely separate spore membranes already formed by precipitation within the cytoplasm. This is made especially clear at the angles of the protospores, where the membranes frequently cut across the corners, leaving small portions of the cytoplasm which do not enter into the formation of any spore (fig. 31, *b*).

MATURATION OF ZOOSPORES.—Although the protospores quickly round off and separate from each other, they remain in the position occupied before segmentation. Consequently the mass of young spores retains the shape of the coenocyte from which it was derived, leaving the central vacuole unoccupied as before segmentation, as in fig. 28, which shows the condition of the great majority of the segmented cysts observed. In such sporangia the young spores are usually regular ovoid cells (fig. 33), without the differentiation of parts characteristic of the mature spore. Only rarely were fully matured zoospores which had moved out into the cavity of the cyst found in the sections studied. In such ripened spores there is a conspicuous differentiation into anterior and posterior ends (figs. 35, 36). In the posterior end is collected the larger part of the cytoplasm with the starch grains, if any be present, while the anterior end appears highly vacuolate in fixed preparations on account of the removal of the pigment which occupied it during life.

In no case was I able to assure myself that cilia were present in the section studied, although I thought I saw them several times. This was probably due to imperfect fixation, since the chromacetic acid used is not as well adapted for preserving such structures as some killing fluids which might have been used had it been possible to experiment on the ground. In zoospores fixed in osmic fumes, after liberation the cilia were of course clearly shown (fig. 37), and in these, as well as in many of those on the sections (fig. 36), there was a conspicuous deeply staining body at the base of the cilia such as has been found in zoospores of many other forms. In many of the spores, especially those a little over-

stained, one or sometimes two delicate connections could be seen between this basal body and the nucleus (fig. 34). The origin of the basal body was not made out. Apparently it appears only during the maturation of the spore, for it was not observed in earlier stages (figs. 32, 33).

The primary nucleus

Although the youngest cysts observed are many times larger than the zoospores from which they originated, their nuclei show comparatively little enlargement. But they differ somewhat in character from the nuclei of the zoospores in that the concentration of the chromatin, which, as shown above (figs. 32–37), begins in the maturing zoospore, has been completed, forming the karyosome, which is the most conspicuous element of the nucleus. But the karyosomes of the young cyst have not acquired the character of the later nucleoli. From the irregularity of their shape they appear to be merely plastic masses of chromatin (fig. 42). They soon take on the definite spherical form of mature nucleoli, and at the same time probably become firmer, inasmuch as in the later vacuolate stages the rind is strong enough to retain its shape after most of the contents have been withdrawn. The linin reticulum seen in the sporangial segments probably persists on the periphery of the nucleus in the youngest stages, but it loses its affinity for stains and is exceedingly difficult to see satisfactorily. All that can be made out with certainty in most of the nuclei is a few delicate linin strands stretching from the karyosome to the nuclear membrane (figs. 1, 13), or, in optical section, a number of peripheral granules (figs. 2, 12), which probably represent cross-sections of the similar strands that compose the reticulum, but are too faintly stained to be visible in surface view.

No differences between the nuclei of the incipient zoosporangia and of resting spores were detected. From the youngest stages on they undergo the same development, which in one case leads to mitosis and in the other to shriveling preparatory to the long dormant period.

The most conspicuous of the changes in the nucleus is its increase in size. From 4 or 5 μ it grows with the cyst until it may reach

the enormous size of 50–60 μ (figs. 15, 45). This size, however, is attained only in the largest zoosporangia. The nuclei of the resting spores are never so large as those of the zoosporangia, which themselves vary greatly, being roughly proportional to the cysts in which they occur. In extremely small cysts the nucleus may never exceed 15 μ, though few are smaller than 20 μ at maturity. In the largest nuclei the increase in volume during growth is almost 10,000 fold. There are but few organisms in which any single nucleus grows to such an extent without division, but *Rhodochytrium* is by no means unique in this respect. In *Synchytrium*, by reason of the minuteness of the zoospores, the increase is very much greater, amounting sometimes to 50,000 fold. In some of the cycads, especially *Dioon* (CHAMBERLAIN 5), the increase in volume must be nearly as great, since the mature nuclei reach 500–600 μ in diameter. The nuclei of some animal eggs, for example *Dytiscus* (DEBAISIEUX 7), also show great increase in volume, but not so much as in the plants just cited.

For the study of the vacuolation of the nucleolus, *Rhodochytrium* and *Synchytrium* probably afford better opportunities than any other organisms, although an analogous process occurs in many plants. Occasionally in *Rhodochytrium* a single central vacuole appears to increase in size until only a thin rind of stainable substance remains. In other cases the whole nucleolus becomes honeycombed with numerous small vacuoles (fig. 46), which later coalesce (fig. 47) into a large central cavity (fig. 44), which continues to increase in size until finally the old nucleolus, originally a karyosome, becomes a plasmosome, collapses (fig. 45), disintegrates, and finally disperses in the cytoplasm during mitosis.

Intimately connected with the history of the nucleolus, and in many ways perhaps even more interesting, is the behavior of the chromatin. As may be seen from figs. 2, 12, 42, the whole of the chromatin is at first concentrated in the karyosome, and from it all of the chromatin of the primary nucleus is derived. While the nucleus is still comparatively small, vacuoles begin to appear in the center of the karyosome (figs. 3, 13, 14), and the characteristic irregular masses of chromatin begin to fill the nuclear cavity. As

in *Synchytrium*, these are most abundant in the vicinity of the nucleolus (karyosome), frequently touching it. Closer examination will often reveal many in the act of budding out from it (figs. 43, 47). During the growth of the nucleus there is, of course, an enormous increase in the amount of chromatin it contains. This increase of the chromatin probably takes place both in the nucleolus during its growth and in the free chromatin of the nuclear cavity. But the withdrawal of the chromatin from the nucleolus must be more rapid than its formation therein, since the vacuolation of the nucleolus increases with age. The linin reticulum, which, as has been seen, loses its affinity for stains in the young cysts, never reappears in the primary nuclei. The chromatin, as it is withdrawn from the karyosome, does not seek the nuclear membrane, but is distributed through the nuclear cavity. In the early stages of growth the chromatin spherules are often connected by indefinite strands of linin, which anastomose to some extent through the nuclear cavity (figs. 3, 14, 44). But in many of the nuclei (fig. 43) such linin connections never appear, and in any case they disappear before the nucleus reaches its full size. In mature nuclei (fig. 45) the chromatin appears as amorphous, almost flocculent, spheroidal masses scattered through the nuclear cavity, singly or in loose chains. The amount of chromatin and the size of its masses vary considerably in different nuclei. In some cases there are relatively few large globules, while in others the chromatin, in a comparatively fine state of division, almost fills the cavity of the nucleus. The small intensely staining granules, which are so conspicuous against the membranes of the primary nuclei of *Synchytrium*, are seldom observed in *Rhodochytrium*, but in some instances (fig. 57) were as prominent as in *Synchytrium*.

The peculiarities of the primary nucleus characterize to a large extent the nuclei of the binucleate and tetranucleate stages, but gradually disappear as the nuclei become smaller, until, from about the 32-nucleate stage on, the nuclei resemble those commonly found in other organisms. Except in the very latest stages, however, both the chromatin granules and the linin connections are coarser than in most nuclei.

Mitosis

There are two types of mitosis in *Rhodochytrium*. The first type occurs in the earlier divisions of the zoosporangium, while the second is found in the last divisions before sporulation. They are not, however, to be considered as distinct, for they merge into each other.

No evidence of a reduction division was found. Nowhere were nuclei seen in fours, as would be expected after reduction; and while the chromosomes are difficult to count accurately, I feel sure that their number was approximately the same in the last divisions as in the primary mitosis. They are extremely difficult to count, however, because they are usually close together and often surrounded by starch grains. For this reason it was not possible to count the chromosomes of as many spindles as would have been desirable, nor to insure exactness in the cases counted. In all of the cases where counting was attempted, however, the number was no smaller than 8 nor larger than 10.

The assembling of a series of stages of mitosis is an exceedingly tedious task. As already stated, coenocytic cysts of any sort are comparatively rare. Those in mitosis are of course rarer still. It is doubtful if one cyst in a thousand of those observed showed dividing nuclei. The anaphases and telophases are particularly difficult to find. It was not possible, therefore, to examine a large number of figures of the different stages. But inasmuch as the spindles found form a concordant series, it is believed that the account given accurately describes the process.

MITOSIS OF THE FIRST TYPE.—The typical mitosis of the first type is the division of the primary nucleus, but the second and third mitoses are so similar that for purposes of description they may be said to be identical. Drawings from all of these have been used in the plates indiscriminately, but they may be identified, if desired, by the explanation of the plates.

Spindle formation.—The first indication of approaching mitosis consists in the appearance of kinoplasmic fibers among the masses of chromatin in the nucleus. The change shown in fig. 48 is so slight that it would hardly have been detected had not the other nuclei of the cyst been already far advanced in spindle formation,

thereby drawing attention to the laggard. Coincident with the appearance of these kinoplasmic fibers the chains of chromatin usually break up, and the individual masses become more definitely spherical, karyosome-like structures. In a nucleus a little further advanced the fibers have become more abundant and permeate all parts of the nuclear cavity (fig. 49), and on some of them are seen small deeply staining granules whose origin, fate, and function are not altogether clear to me.

From the very first the position of one of the poles of the future spindle can be recognized in the focus of certain of the fibers (figs. 48–50). Curiously enough, however, the other pole does not seem to appear until somewhat later, so that the young spindles show a considerable difference in the two poles, one being more fully formed than the other (fig. 51). This is such a peculiar phenomenon that one is strongly inclined to believe, when he finds such a nucleus, that he has overlooked the opposite pole on another section (most of the spindles are of course somewhat oblique), but careful search almost invariably failed to reveal it. Fig. 50, which is a sagittal section of a primary nucleus, shows perhaps the extreme of this condition; notwithstanding the strong development of kinoplasmic fibers in the part of the nucleus drawn, they were entirely absent from the other parts. It is quite possible that the spindles seen in these stages were unusual, but the occurrence of the unipolar condition in different pieces of material killed in different years has convinced me, against my prejudices, that this is a normal and usual method of spindle formation.

Such a drawing as fig. 50 resembles the prophase in the Ascomycetes, in which the linin strands containing the chromatin radiate from one side of the nucleus. There are, however, important differences between the two. The polarity of the ascomycetous spindle is determined by the presence of centrosomes attached to the nuclear membrane, but in *Rhodochytrium* no centrosomes are visible, and the pole does not necessarily touch the nuclear membrane at all. The origin of the bipolar condition is entirely dissimilar. In the Ascomycetes the two centrosomes, derived from the fission of one, separate and migrate to opposite sides of the nucleus, each carrying with it its quota of fibers with attached

chromatin. But in *Rhodochytrium* the second pole is formed, like the first, by the convergence of certain fibers to a point. In nuclei a little older than that shown in fig. 50, some of the kinoplasmic fibers can be seen to intersect at points more or less directly opposite the first pole. There are usually two or three such points (fig. 51), from each of which a few fibers radiate. In later stages one of these focal points becomes more prominent than the others, until ultimately it becomes the second pole of the spindle, as prominent and definite as the first.

In the fully formed spindle the larger proportion of the fibers of course stretch from pole to pole, but in the early stages the rays from each pole appear as an independent fascicle radiating from the focus, with little regard to the position of the opposite pole. The vestiges of this condition may be seen in fully formed spindles, in which many of the acicular mantle fibers stretch straight by the equator of the spindle, intersecting those from the opposite pole (figs. 52–54). Not infrequently a few fibers center in the pole and do not enter into the formation of the spindle, but radiate into the nuclear cavity. In one instance such radiations were so numerous as to give the appearance of a conspicuous aster (fig. 53). But comparison with the opposite pole shows that the effect here produced is largely accidental. Nothing similar was seen elsewhere.

Chromosome formation.—The differentiation of the chromosomes, in my material, is a much more difficult matter to follow than the formation of the spindle. Of the masses of chromatin which are distributed throughout the nuclear cavity, part remain free and part become connected with the developing spindle fibers. In addition to these, some of the spindle fibers, especially in the early stages, are studded with smaller chromatic granules whose significance, as stated above, is obscure to me. At one stage of the investigation I was inclined to believe that these were used in the formation of the chromosomes, but further observation has led me to the conclusion that it is the large chromatin masses which give rise to the chromosomes. Whether the chromosomes are derived exclusively from the latter is not certain, but such figures as no. 51 show at least that some of them are utilized in chromosome formation.

The formation of the chromosomes, though it presents certain striking peculiarities, conforms in its essential features to the process usually found in dividing nuclei in other organisms. As is not unusual, spindle formation and chromosome formation, being in a sense unconnected processes, may go on side by side with a certain degree of independence, so that in two nuclei of the same age one may have the more mature spindle, while the other has advanced further in chromosome formation (figs. 52, 53).

Spirem formation will be understood by a glance at fig. 51. Between those chromatin spherules which lie in the equatorial region of the nascent spindle there arise connecting bands of linin, forming an irregular spirem. At first the stains differentiate the chromatin and the linin elements, but in later stages the spirem stains homogeneously like other spirems. In the beginning its position may not be so definite, but as it contracts it comes to lie wholly within the spindle (fig. 52). After some further contraction it segments into chromosomes in the usual way (figs. 53, 54).

Only a small portion of the chromatin of the primary nucleus is utilized in the formation of this spirem. On the dissolution of the nuclear membrane the remainder is cast out into the cytoplasm. There is no indication of any difference between those chromatin masses which are cast out and those which enter into the spirem, nor of any principle of selection other than that occasioned by the mere position of the masses which are utilized. Sometimes the masses of discarded chromatin persist for some time as deeply staining globules in the cytoplasm (fig. 22), but more often they lose their affinity for stains before the nuclear membrane breaks down and cannot be followed in later stages.

During metaphase the spindle, which previously may have been shorter than the diameter of the nuclear cavity in which it lay (fig. 53), begins to elongate, piercing the membrane (fig. 54), and later, as the membrane weakens preparatory to dissolution, distorting the nucleus (fig. 55). The only anaphases seen were of the first type, occuring in the fourth mitosis. Apparently the chromosomes are drawn away from the equator in the usual way (fig. 56). No stages showing the formation of the membranes of the daughter nuclei were seen in spindles of the first type, but two

recently divided binucleate cysts were found. Their chromatin strands (fig. 57) still showed by their orientation the position of the chromosomes from which they had been derived. As stated above, the position of the mother nucleus is still clearly indicated by a starch-free area in the cytoplasm.

No centrosomes or asters, except the pseudoaster above noted, were seen in connection with any of the spindles. The poles are very sharp, without any surrounding zone of denser cytoplasm in which a centrosome might have been concealed. There is no indication that astral bodies have any part in the formation of the nuclear membrane, as in *Synchytrium decipiens*.[3] While but very few of the critical stages were seen, it seems evident that, if there were any such conspicuous asters as in that plant, they would certainly have appeared in the preparations studied.

In the intermediate mitoses, spindle formation conforms in a general way to that in the primary nucleus, but the metaphases (figs. 58, 59) are so different that at first sight they would seem to be of an entirely different type. The differences, however, are not so great as would appear. In the smaller nuclei nearly as great an amount of chromatin is used in the formation of the chromosomes as in the larger. Their spirems are therefore much larger proportionately, and, instead of lying within the spindle, stretch nearly across the nuclear cavity. Sometimes such spindles show a considerable amount of chromatin which is not utilized in the formation of the chromosomes, but is cast out, as in the earlier divisions. Frequently, however, all of the chromatin goes into the spirem (fig. 59). The karyosome, which is so strongly developed in the primary nuclei, becomes gradually less and less prominent in later nuclei, until in the many-nucleate cyst the chromatin assumes the condition of a typical reticulum, although it is not finely divided, but remains in rather large masses which are connected by coarse linin strands (figs. 27, 29). In consequence of the different dispositions of the chromatin in these nuclei, the residual chromatin cast out during their mitosis does not take the form of large spherules, but is finely subdivided (fig. 58). Such a condition was also seen

[3] *S. taraxaci* is without karyodermatoplasts according to the recent results of BALLY (Jahrb. Wiss. Bot. **50**: 110. 1911).

in one primary nucleus, in which case the residual chromatin was much more abundant than in the smaller nuclei.

MITOSIS OF THE SECOND TYPE.—The second type of mitosis is limited to the last few divisions before sporulation. Unfortunately nearly all of the mitoses of this type that were found occurred in cysts packed full of starch, which greatly interfered with observation.

The difficulties occasioned by this cause were especially serious in studying the prophases. In the cyst from which the figures of prophase were taken, all stages of prophase were certainly present, but could not be made out satisfactorily. The nuclei of the upper half of the sporangium had already passed into the metaphase, while those in the rhizoidal end were still in the vegetative condition (fig. 60), and above them all transitions to metaphase were present. As far as could be determined, these prophases were similar to those of the smaller nuclei of *Synchytrium*. A spirem is formed which in this case involves but little change from the vegetative condition. This then shortens and thickens until it comes to occupy only the equatorial region of the nucleus (fig. 61). The spindle then appears, whether as a new formation or as a metamorphosis of linin strands as in *Synchytrium* could not be determined.

The chromosomes in this type of mitosis are small and spherical (fig. 62), but apparently stretch out somewhat in fission, for at the poles in telophase they are distinctly oblong (fig. 63). In early telophase they are bunched together in a compact mass resembling the familiar "daughter star," but later begin to spread out (fig. 64) and assume irregular shapes (fig. 65), while vacuoles of karyolymph begin to appear among them, soon producing the characteristic vegetative nuclei (fig. 66). As may be seen from the figures, these stages are practically similar in all respects, save in the absence of cell plate, to the familiar anaphases and telophases of the higher plants.

Amitosis

Amitosis, which forms such a conspicuous feature of the cytology of *Synchytrium*, is almost absent from the zoosporangia of *Rhodochytrium*, or at least from the material studied. The nuclei of a few

cysts, however, are in such a condition that it seems hardly possible to interpret them as sister products of mitosis. Their chromatin assumes the condition of an extremely long and complicated spirem, which winds not only around the surface of the nucleus but fills its cavity (figs. 67, 68). Their shape is extremely irregular. The largest have developed pseudopodium-like outgrowths, which appear to have been constricting off into daughter nuclei. With these large nuclei are a number of small ones, apparently the results of the process. While the mere irregularity in the outlines of these nuclei would not in itself be conclusive evidence that they were dividing amitotically, the great diversity in the sizes of adjacent nuclei would be difficult to account for on any other hypothesis. For in *Rhodochytrium*, as in coenocytes generally, the mitoses are simultaneous, and the daughter nuclei are of approximately equal sizes (figs. 22–27). It is evident that such a process could not normally give rise to irregularities in either number or size of the resultant nuclei.

There is no indication, however, that amitosis is a normal process in the zoosporangia of *Rhodochytrium* as in *Synchytrium*. It gives rather every indication of being a pathological phenomenon.

Cytological comparisons

PRIMARY NUCLEUS.—The primary nuclei of *Rhodochytrium* are certainly very peculiar; indeed, if the cytology of *Synchytrium* were not known, we should say they were unique. But when mature they are strikingly similar to those of *Synchytrium*, or at least to those of *S. decipiens* and *S. puerariae*. The conditions sometimes found during the early portion of the growth period, however, are not paralleled in *Synchytrium*. The early stages of *Synchytrium* are very similar to the mature nuclei, but in the young nuclei of *Rhodochytrium* the chromatin spherules are often suspended on anastomosing strands of linin within the nuclear cavity (figs. 3, 14, 44). This condition is evidently less removed from the typical peripheral chromatin-linin reticulum of most nuclei than are the mature nuclei or those of *Synchytrium*.

The irregular masses of chromatin in the primary nucleus of *Synchytrium* are termed by KUSANO (18) secondary nucleoli. He

shows, what I have myself observed, that they may pass through a process of vacuolation accompanied by the extrusion of chromatin analogous to that of the primary nucleolus. In *Rhodochytrium* such secondary vacuolation occurs but rarely, though some of the largest chromatin masses may break up in this way (fig. 49). But, as was shown in the account of mitosis, a large proportion of the chromatin spherules suffer the same fate as the old nucleoli, primary and secondary, of *Synchytrium*, namely dissipation in the cytoplasm. There is, moreover, a great variation in the size, composition, and behavior of the secondary nucleoli in *Synchytrium* (see KUSANO **18,** p. 94), some of them (the earlier and smaller) being almost, if not entirely, pure chromatin, and undergoing but little change in preparation for mitosis; while others (the later and larger) are plasmosomes with but little chromatic material. There is, therefore, no question but that the chromatin masses of *Rhodochytrium* are homologous to secondary nucleoli, but it does not seem advisable to use that term in describing them, since there is no distinction between those which form the chromosomes of the spindle and those which perish.

MITOSIS.—The first mitoses of *Rhodochytrium* and *Synchytrium* are not so similar as are the primary nuclei, but they are of the same general type. Although very different from those found in most organisms, the first mitosis of *Rhodochytrium*, like the vegetative condition of the primary nucleus, is not so widely aberrant as that of *Synchytrium*. Neither STEVENS nor KUSANO was able to obtain an altogether satisfactory series of the prophases of the primary mitosis, and their figures do not supplement each other, but conflict to a certain extent. Both observed, however, a marked and peculiar production of fibers, STEVENS through the whole cavity of the nucleus, and KUSANO especially in the region of the old nucleolus after the dissolution of the membrane. While the conditions found by these writers in *Synchytrium* differ greatly in detail from those in *Rhodochytrium*, the fibers would seem to be comparable to those seen in the early prophases of the present plant. If this interpretation is correct, the fibrous stage in *Synchytrium* is not to be homologized with a spirem, but is rather a phase of spindle formation. The differentiation of the chromosomes,

which neither of these writers was able to observe, would on this assumption be a distinct process. While it cannot be predicted that in the differentiation of the chromosomes *Synchytrium* will be found to resemble *Rhodochytrium*, it is clear that in the formation of the spindle there is considerable analogy.

While the metaphases, and probably the prophases as well, of the second type of mitosis are similar to those of *Synchytrium*, this has no particular significance, since they present no peculiarities, but are similar to those of many other organisms. The telophases, however, differ considerably from those of *Synchytrium*, both in general form and in the absence of the conspicuous kinoplasmic asters, *karyodermatoplasts*, which in *Synchytrium decipiens* and *S. puerariae* form the nuclear membranes of the daughter nuclei. These structures remain, therefore, peculiar to these species.

KARYOLYMPH.—The large primary nuclei, of course, are cut into several sections by the microtome. The central section of such a nucleus presents an appearance which would hardly be recognized by the uninitiated, for it looks at first sight like a hole in the cytoplasm of the parasite. It is surrounded, however, by the nuclear membrane and contains some of the amorphous masses of chromatin and perhaps a part of the nucleolus. But sometimes the whole nuclear cavity is filled with a frothy mass similar to that noticed by KUSANO in *Synchytrium* after fixation with Keiser's fluid. It appears to be, what KUSANO interpreted it, a precipitation from karyolymph. I have not figured it because it is inconstant in occurrence and imperfectly understood.

It should be noticed here, however, that the karyolymph may very likely play a much more important rôle in cell physiology than is at present assigned to it by cytologists. It is dismissed with a sentence in such texts as WILSON'S *Cell*, because our knowledge of it is practically nil. Yet, ignorant as we are, a little reflection will convince us that it must be of some consequence to the cell. On the amount of karyolymph depends the size of the nucleus, for it is in reality merely a vacuole of karyolymph around which is stretched the chromatin reticulum. It is a well-known fact that by some means the size of this vacuole is maintained with slight variation in the cells of a given tissue. We know further that when

by any abnormality the amount of chromatin is increased, as when a nucleus passes through the prophases of mitosis but fails to divide, the karyolymph is proportionately increased. The characteristic phases of the nucleus, vegetative and mitotic, are marked off from each other principally by the appearance and dispersal of the karyolymph. Indeed, it is a general rule that whenever the karyolymph is absent, the anabolic activity of the cell is suspended. The characteristic condensed condition of sperm nuclei is another illustration. The shriveling of the nuclei of the resting spores in *Rhodochytrium* above described is due to the partial disappearance of karyolymph when growth ceases and the dormant period is entered upon.

SEGMENTATION.—In regard to the process of segmentation, the uncertainties encountered in *Rhodochytrium* are largely duplicated in *Synchytrium*. HARPER (12) reported that segmentation occurs by the formation of cleavage furrows, which begin to penetrate the cytoplasm at a relatively early stage in the multiplication of the nuclei. KUSANO (18) found that while some cysts undergo progressive cleavage, as described by HARPER, others show simultaneous segmentation by the precipitation of membranes around the segments. My own observations, like KUSANO'S, showed both of these methods of segmentation, but in my material the progressive cleavage described by HARPER was infrequent. The apparent duplication of segmentation recalls the double contraction reported in various phycomycetes and certain algae, such as *Hydrodictyon* (KLEBS 14, TIMBERLAKE 30). But it is not easy to correlate the accounts of observations on living and on fixed material, and for that reason the writer finds himself unable to interpret the phenomena satisfactorily.

Alga or fungus?

Having examined the morphology and cytology of the plant, we may proceed to consider its relationships. Since it is an obligate parasite without chlorophyll, one naturally wonders how it was ever referred to the protococcoid algae. On a superficial examination certainly, it would appear that the plant is no alga but a chytrideaceous fungus. The first question that arises, therefore, is

whether *Rhodochytrium* is an alga or a fungus. As will be seen, the answer depends not so much upon any interpretation of the facts of the case, as upon the point of view of the student.

Among the Chytridiales, *Entophlyctis*, of the family Rhizidiaceae, is strikingly similar to *Rhodochytrium* in gross morphology. Both are characterized by an external button connecting by a narrow neck with the main body of cyst. The rhizoidal system, if not exactly of the same appearance in the two cases, is of the same type, and the differences may be supposed to be due to the character of the substrata, which in one case is the soft protoplast of an alga and in the other the tough vascular bundle of a seed plant. The life cycles are identical; both start from a free swimming zoospore that penetrates the host, giving rise to an internal ampulla which on maturity becomes either a resting spore or a zoosporangium. Altogether *Entophlyctis* is so similar to *Rhodochytrium* that the comparison is exceedingly suggestive.

Nevertheless, there does not seem to me to be any good reason for connecting *Rhodochytrium* and *Entophlyctis*. The comparative anatomy of the Rhizidiaceae would seem distinctly to forbid such an idea. Within the family Rhizidiaceae there are apparently all transitions from purely epiphytic parasites with as little penetration as possible, to complete endoparasites. At the beginning of the series may be placed *Rhizophidium brevipes*,[4] which barely penetrates the wall of its host, without putting out any rhizoids to gather nutriment. Further stages are shown by various species of *Phylactochytrium*, which not only have extensive rhizoids, but develop a small basal portion of the plant body itself within the host. In *P. equale* the internal portion of the body becomes as large as the external. From this condition it is an easy step to *Entophlyctis* by the enlargement of the internal portion at the expense of the external, with consequent transference of the sporogenous function. This has every appearance of being a natural phyletic series. In it the parasitic mode of life would appear to

[4] *Harpochytrium* is even more surely an epiphytic parasite, since it does not penetrate its host at all, being merely attached to its wall; but it is not used in the present comparison because its relationships have been subject to some difference of opinion among different observers. WILLE (**33**), for example, believes that it is a colorless member of the Protococcoideae.

have been developed from an epiphytic ancestry, while endophytism did not appear until later.

In contrast with this group, *Rhodochytrium* seems to have been derived from organisms which acquired the endophytic habit of life before any real dependence on their hosts was established.

Moreover, the zoospores of *Rhodochytrium* appear to differ fundamentally from those of the Chytridiales. In most of the latter there is but one flagellum, which is often trailed along behind and imparts a weak jerky motion to the spore. In the genera with biflagellate zoospores the flagella, in most cases at least, are of the same type, and usually spring from different portions of the body.[5] Sometimes also the spores put out pseudopodia and move about in amoeboid fashion. In *Rhodochytrium* the zoospores are capable of no such motion, but maintain the integrity of their shape with slight variation throughout their period of activity. The cilia, which are anterior, are more highly specialized structures and maintain a rapid vibration which propels the spore with the steady motion characteristic of algal zoospores in general, to which those of *Rhodochytrium* correspond in every important particular, save in the absence of chlorophyll.

But the nature of the parasitism of *Rhodochytrium* indicates a very considerable degree of departure from the algae. An obligate parasite which has established definite relations with specific hosts, even though its different races show no morphological modification, is certainly far from a typical alga. The loss of plastids is an important characteristic of the fungi, but the presence of starch grains looks back toward the algae. Though starch has been reported in several fungi, and some of them contain certain carbohydrates which give the starch reaction with iodine, such as starch cellulose ("lichenin"), there is no well authenticated instance of the occurrence of definite grains of starch in any undoubted fungus.

Turning now to the algae along the lines suggested by LAGERHEIM'S paper, we find among the protococcoid algae a number of

[5] In a paper to be published almost concurrently with this (Ann. Botany, January 1912), the proof of which I have seen through the kindness of the author, Dr. J. T. BARRETT, it is shown that the zoospores of several species of *Olpidiopsis* have two flagella *springing from the same point*, while other species of the same genus are reported as uniflagellate.

very interesting endophytes or "*Raumparasiten*," which have been made known principally by the researches of KLEBS (13). The climax of this series is found in *Phyllobium dimorphum*, which penetrates dying leaves of *Lysimachia nummularia*. Its adult body is strikingly similar to that of *Rhodochytrium*. There is a long empty neck, with an external cellulose button connecting the internal cyst with the wall of the zoospore from which it developed, just as in *Rhodochytrium*. In its most typical development this plant is confined to the vascular bundles of its host, into which it penetrates very much as does *Rhodochytrium*. It sends out, moreover, numerous interlacing rhizoids, which follow along the bundles for considerable distances, and even extend up their branches. On germination the resting cysts give rise to biciliate zoospores which conjugate as in *Rhodochytrium*, except that there is a slight sexual differentiation, microzoospores and megazoospores being formed in different cysts. The cysts and the zoospores have abundant chlorophyll, but haematochrome is also present in considerable amounts in some stages of the life cycle. Little is known of the finer structure or cytology[6] of this plant, but, so far as one can judge from the evidence available, it is remarkably close to *Rhodochytrium*. The most important difference between them would seem to be the presence of chlorophyll in the one and its absence in the other. OLTMANNS (**21**, pp. 322 ff.) believes that these forms belong to a natural series. He agrees with LAGERHEIM that *Rhodochytrium* is an alga, saying "while the first named genus [*Phyllobium*] cannot be considered more than an endophyte, as we have already clearly demonstrated, *Rhodochytrium* is one of the rare examples of an alga which has lost its chlorophyll on account of parasitism."

It will be seen, therefore, that the decision as to whether *Rhodochytrium* is an alga or a fungus depends upon the criteria by which the line between them is to be drawn. If the question is to be settled by definition, we should follow VUILLEMIN (**32**) and call it a fungus, for it would be very difficult to frame a definition of the fungi which would exclude *Rhodochytrium*. This position is also

[6] OLTMANNS states, on the basis of unpublished observations by GRUEBER, that the cyst is uninucleate.

taken by LINDAU (**20**), who excludes it from the algae on account of the absence of chlorophyll. If, on the other hand, the matter is to be decided by the relationships of the plant, it is clear that since its nearest affinities are with undoubted algae, *Rhodochytrium* must be considered an alga. It is not a matter of great consequence whether such an organism is considered a fungus or an alga, so long as its real affinities are recognized. But in the case of *Rhodochytrium* it will probably be more convenient to consider it with the algae than with the fungi, since it can be satisfactorily approached only from the algal side.

Evolutionary inferences

But although *Rhodochytrium* is to be considered the extreme of an algal series and not a near relative of any of the Archimycetes, the phyletic position of the Phyllobiae, taken as a whole, remains to be considered. We have here a series of endophytes culminating in a colorless parasite. Does this line of evolution end blindly, or do these forms furnish the clue to the origin of some fungal group? Nearly forty years ago, before *Phyllobium*, *Rhodochytrium*, and *Endosphaera* were discovered, COHN (**6**) recognized the general similarity of his newly discovered *Chlorochytrium* to *Synchytrium*, and suggested that the two were phylogenetically connected.

There are now known far more points of similarity in gross morphology between the different genera of the Phyllobiae and *Synchytrium* than those which induced COHN to make the comparison. Indeed, could one construct a plant with a combination of characters from the different genera, he would have a very satisfactory transition to *Synchytrium*. Such a hypothetical plant would be an obligate parasite definitely limited to specific hosts, like *Rhodochytrium*. But it would have no rhizoids, retaining rather the simple spheroidal form of *Chlorochytrium* and *Endosphaera*. It would have simultaneous segmentation like *Rhodochytrium*, but the segments would become sporangia rather than zoospores, as in *Endosphaera*, which has substantially the same method of reproduction as *Synchytrium*, except that the swarmers conjugate, while in *Synchytrium* no sexual process is known. It would have lost its plastids, and instead of having chlorophyll

would be pigmented with haematochrome. Should such a plant be discovered, the probabilities are that it would be placed in the Synchytriaceae rather than among the Phyllobiae, where by hypothesis it belongs.

But it must be recognized that the comparison fails utterly at certain points. The germinating zoospore of *Synchytrium* does not form an external button on the surface of its host, and the zoospores are of different types, as shown above. These matters are regarded by some as fundamental criteria of relationship. PETERSEN (23) considers that the presence of an external button in the Chytridiales is clear evidence that they have originated from the filamentous Phycomycetes. But this contention would lose its force if applied to *Chlorochytrium* and *Rhodochytrium*, for these would hardly be regarded by anyone as reduced Siphomycetes. The number of flagella borne by the zoospores is used as a fundamental basis of classification by LOTSY and by VUILLEMIN (32), who regard the genera with biflagellate zoospores as entirely distinct from the other Archimycetes, and classified with them merely because of accidental similarities in form, using as an example *Myzocytium*, which, however, appears distinct from the Chytridiales for other reasons as well. But the Javanese genus *Woroninella* was separated from *Synchytrium* almost entirely on account of the possession of biflagellate zoospores. In all other characters, including the large primary nucleus, it seems to be exceedingly close to *Synchytrium*. Our present information concerning *Woroninella*, which is all contained in a brief description without figures (RACIBORSKI 24), is too meager to enable us to judge whether it is transitional between *Rhodochytrium* and *Synchytrium*. But the description of *Woroninella* goes far to remove those objections to connecting the two that are based on the differences in the zoospores (see also footnote p. 163).

As has been pointed out above in the detailed cytological comparisons, there are some very striking resemblances in cytology between *Rhodochytrium* and *Synchytrium*. Some of these are peculiar to the two genera, being unknown in other organisms. The most conspicuous and perhaps the most significant of these is the enormously overgrown primary nucleus. It is evident that

these are truly unicellular organisms devoid of nuclear as well as cell division until the beginning of the reproductive period. The single cell which composes the plant body does not show any notable specialization in its cell organs, but it reaches a size which is exceeded only by a very few of the largest infusorians, while no nuclei of anything like equivalent size are to be found elsewhere among the Protista. The resemblances in these primary nuclei are not merely superficial, but are emphasized by detailed comparisons of their structure. Though their mitoses differ somewhat in detail, they also are certainly analogous in many respects.

These cytological resemblances, coupled with the general similarity in gross morphology and the tendency toward parasitism so evidently manifest in the Phyllobiae, are certainly very suggestive. It is difficult to imagine that such peculiar cytological features originated independently. If the cytology of the other members of the Phyllobiae and of the genera closest to *Synchytrium* should fall into line with the evidence now available in *Rhodochytrium* and *Synchtrium*, it would make a strong case in favor of a phyletic relationship between the two groups. But it would afford no reason for supposing them *closely* related, for *Synchytrium* appears to occupy an isolated position. The gap which separates it from Phyllobiae would appear to be of ordinal rank, and, at the same time, it is generally recognized that it is far from most other Archimycetes. Nor would it show that *Synchytrium* was derived directly from *Rhodochytrium* or even from Phyllobiae. But it would indicate that these forms may serve as a guide post pointing out the most probable location of the evolutionary path followed by the ancestors of *Synchytrium*.

Summary

Rhodochytrium does not appear in North Carolina until late in the spring; at first zoosporangia are most abundant, but late in the season only resting spores are found.

The cysts are independent, not connected through their rhizoids.

The zoospores are of the algal type and frequently contain starch grains, but are colorless except for the red anterior end; they are

active for half a day or more, but seem to conjugate rarely except when confined in small amounts of fluid.

The nature of the cyst (resting spore or zoosporangium) is determined on infection.

The red pigment which is found at all stages of the life cycle is haematochrome or an allied lipochrome.

Although the three races of *Rhodochytrium* appear to be geographically isolated and affect different hosts, no morphological differences were detected between them.

The germ tubes do not enter the stomata, but penetrate the epidermis at any point, usually in the vicinity of a vascular bundle.

The cysts, both resting and temporary, are uninucleate until full size is attained.

Their rhizoids extend along the vascular bundles, mostly in the phloem elements, which they destroy, but they also send haustoria to the vessels of the xylem.

When mature the resting spores have a two-layered cellulose exospore and a thick non-cellulose endospore; most of the reserve food is in the form of starch; the nuclei are considerably shriveled by the withdrawal of karyolymph.

The starch grains are similar to those commonly seen in the higher plants.

No plastids could be found, the starch grains appearing to be built up directly in the plasma.

The flaring necks of the zoosporangia are stopped by characteristic turbinate or bell-shaped plugs.

During the last mitoses there is a contraction which results in a pseudo-segmentation, but true segmentation appears to be brought about by the precipitation of membranes around the protospores.

There is a deeply staining body at the base of the cilia of the zoospores which is connected with the nucleus.

The primary nuclei, which reach the size of 50–60 μ, have enormous nucleoli and peculiar amorphous masses of chromatin like *Synchytrium decipiens*.

In the first type of mitosis, the spindle, which is usually unipolar at first, is formed from coarse acicular fibers that appear within

the nuclear cavity; it has no connection with the nuclear membrane. The spirem is formed from that part of the chromatin which lies in the equatorial region, the rest being cast out; it is frequently entirely within the spindle.

The second type of mitosis presents no unusual features.

No centrosomes or true asters were seen.

Amitosis is rare and abnormal in the zoosporangia.

Although superficially resembling *Entophlyctis*, *Rhodochytrium* is not closely related to any known Archimycete.

But it appears to be closely related to the Protococcoideae through *Phyllobium*.

The Phyllobiae show considerable similarity to *Synchytrium* in gross morphology.

The cytology of *Rhodochytrium* bears a strong resemblance to that of *Synchytrium*.

These resemblances suggest that *Synchytrium* was derived from protococcoid ancestors.

OHIO STATE UNIVERSITY
COLUMBUS, OHIO

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EXPLANATION OF PLATES XI–XVI

The figures were made with various combinations of Zeiss apochromatic and Spencer achromatic oil immersion lenses with compensating oculars. The magnification of the different figures is given in the description of each. The figures have been reduced one-third in reproduction, canceling the enlargement due to the camera and rendering them the same size as when seen in the microscope. All of the figures, except 16 and 19, were taken from the race of the parasite on *Ambrosia artemisiifolia*.

FIG. 1.—Young resting spores; ×334.

FIG. 2.—Somewhat older resting spore, spreading out irregularly in the tissue; ×334.

FIG. 3.—Cyst in which the basal portion has swollen out, although the protoplast has grown but little; rhizoids not in plane of section; nucleus with numerous spherules of chromatin connected by linin strands scattered through its cavity; ×334.

FIG. 4.—A full-sized resting spore whose wall is beginning to thicken, with that portion of the rhizoidal system which lay in the plane of section; ×334.

FIG. 5.—A portion of the rhizoidal system of a mature cyst, showing its relation to phloem and xylem; ×334.

FIG. 6.—Detail of a haustorium closely applied to a pitted vessel; from a cyst which had surrounded itself with a thick wall, hence the wall around the haustorium; ×334.

FIG. 7.—Two-layered resting spore, showing the shriveling of the nucleus and the cutting off of the rhizoids; ×334.

FIG. 8.—Mature three-layered resting spore; ×334.

FIG. 9.—Starch grains from mature cysts, showing variations in size and shape; ×2000.

FIG. 10.—Starch grains from a mature cyst under polarized light; ×2000.

FIG. 11.—Young zoosporangium with numerous fine strands of cytoplasm in which starch is forming; ×334.

FIG. 11*a*.—Detail from fig. 11, showing formation of starch grains; ×3000.

FIG. 12.—Young zoosporangium; ×334.

FIG. 13.—Young zoosporangium just beginning to swell out; ×334.

FIG. 14.—Young zoosporangium in the stem of the host; plug already developed, although the tubular form is still retained; ×334.

FIG. 15.—Full-sized zoosporangium, showing the characters of the primary cyst; ×334.

FIG. 16.—A typical turbinate plug from the race on *Asclepias pumila;* ×670.

FIGS. 17, 18.—Lamellate plugs; ×670.

FIG. 19.—An unusual form of lamellate plug from the race on *Spilanthes;* ×670.

FIG. 20.—A bell-shaped plug, apparently secondarily filled up; ×670.

FIG. 21.—A bell-shaped plug which is perforate; ×670.

FIG. 22.—A binucleate cyst with part of its rhizoids showing by a starch-free area the approximate size and position of the primary nucleus and the remains of the residual chromatin cast out during the primary mitosis; ×334.

FIG. 23.—The tetranucleate stage; drawn from two sections of a retort-shaped cyst with the bend perpendicular to the plane of section; the sporangium, and especially the vacuole, were therefore larger than is indicated in the drawing; ×334.

FIG. 24.—An 8-nucleate cyst in which the position of the primary nucleus is still clearly indicated by a starch-free area in the cytoplasm; only six nuclei in plane of section; ×334.

FIG. 25.—An oblique section of 16-nucleate cyst in which the nuclei were still bunched in the center; ×334.

FIG. 26.—A small cyst in the 32-nucleate stage; ×334.

FIG. 27.—A cyst with about 128 nuclei; ×334.

FIG. 28.—A segmented zoosporangium, the protospores still retaining the shape of the coenocyte from which they were derived; ×334.

FIG. 29.—A cyst apparently segmenting by cleavage furrows; ×1000.

FIG. 30.—Preliminary contraction resulting in pseudo-segmentation during the last mitoses; ×1000.

FIG. 31.—Segmentation by the precipitation of membranes in the cytoplasm: *a*, a wall just forming; *b*, a portion of cytoplasm left out between the segments; ×1000.

FIG. 32.—A newly formed protospore; ×2000.

FIG. 33.—A protospore rounded off and beginning to show the concentration of the chromatin; ×2000.

FIG. 34.—A protospore overstained, showing the body at the base of the cilia and its connection with the nucleus; ×2000.

FIG. 35.—A young spore with starch grains partly concentrated in the posterior end; chromatin concentrated into a single mass; ×2000.

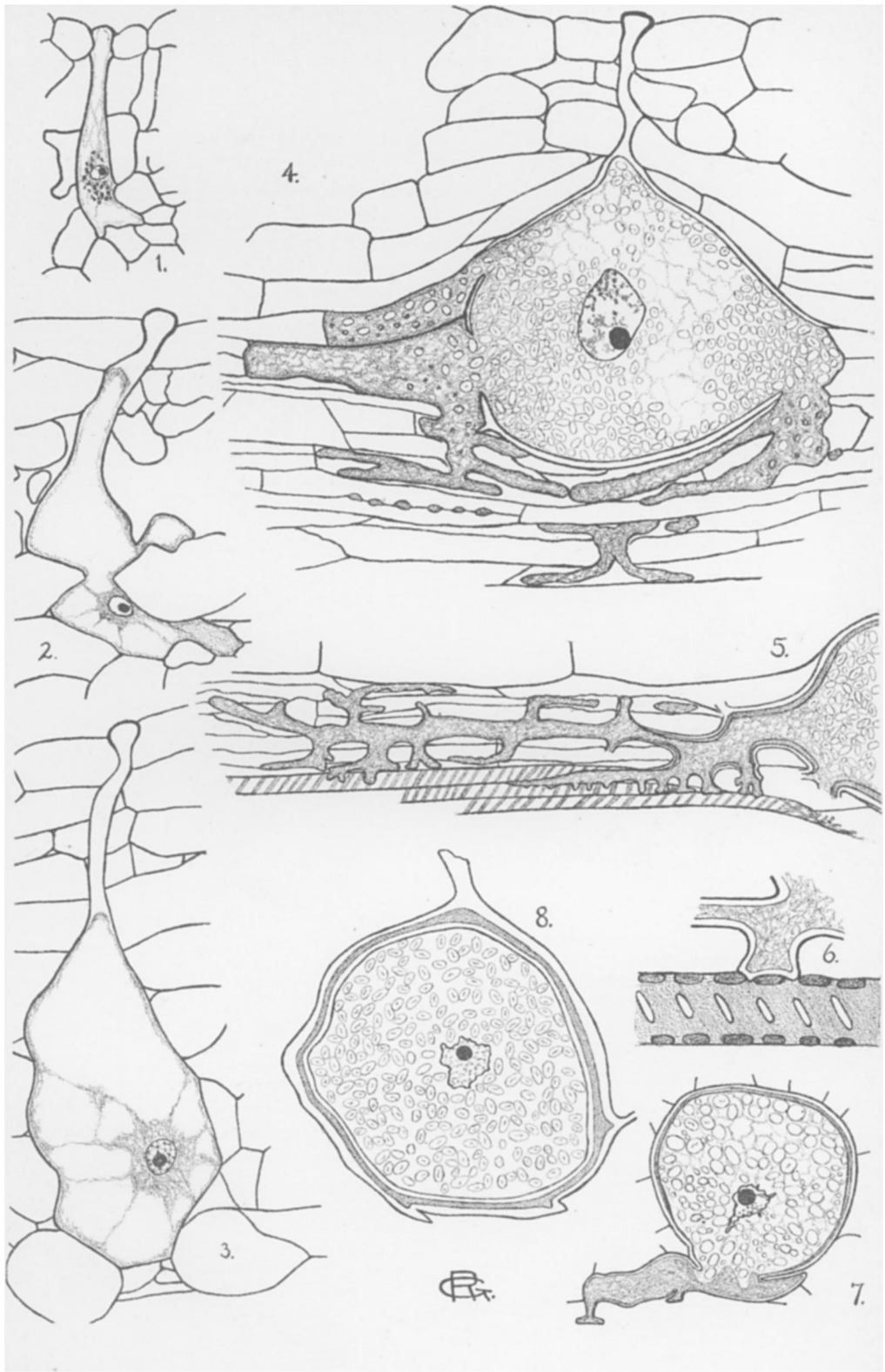
FIG. 36.—Mature spore from a section showing basal body and antero-posterior differentiation of the spore; ×2000.

FIG. 37.—Free swimming zoospore killed with osmic fumes stained with gentian-violet; ×1000.

FIGS. 38–41.—Stages in the conjugation of the zoospores from living material; cilia diagrammatic; the difference in size between the gametes was accidental; there is no differentiation into microgametes and megagametes.

FIG. 42.—A nucleus from a very young cyst; ×2000.

FIG. 43.—A nucleus from a young resting spore; vacuolation of karyosome beginning; few connections between the chromatin spherules; ×2000.



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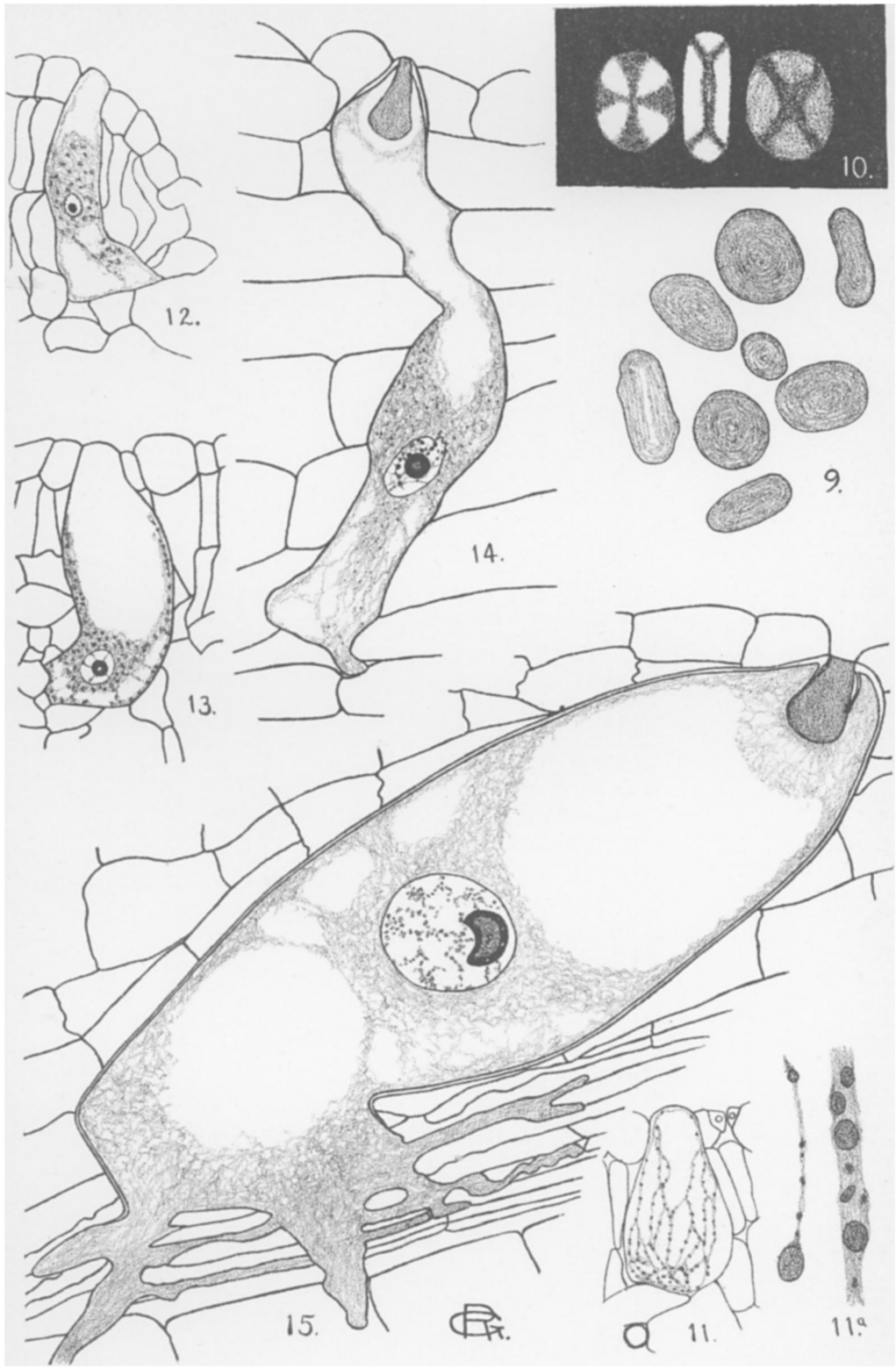

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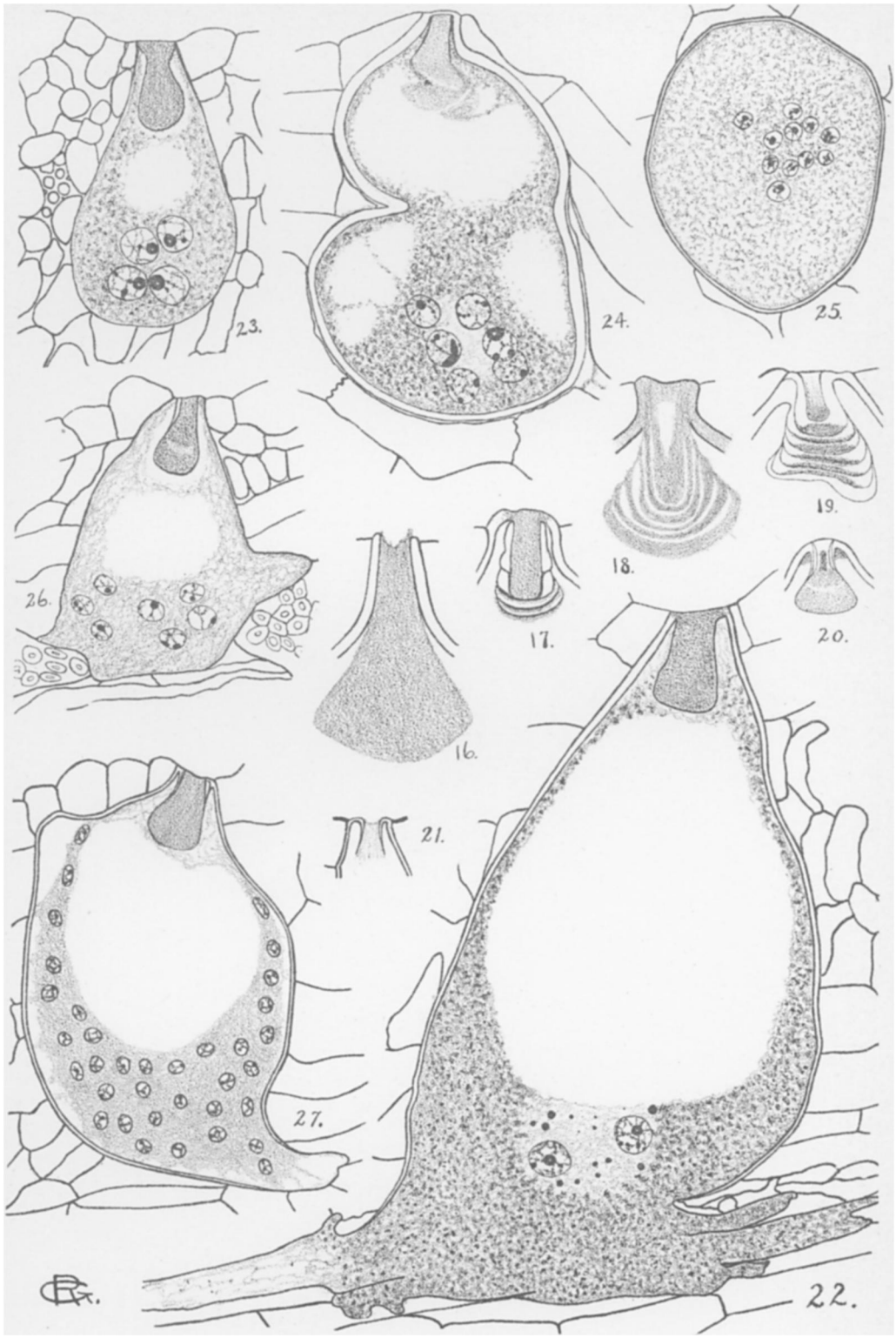
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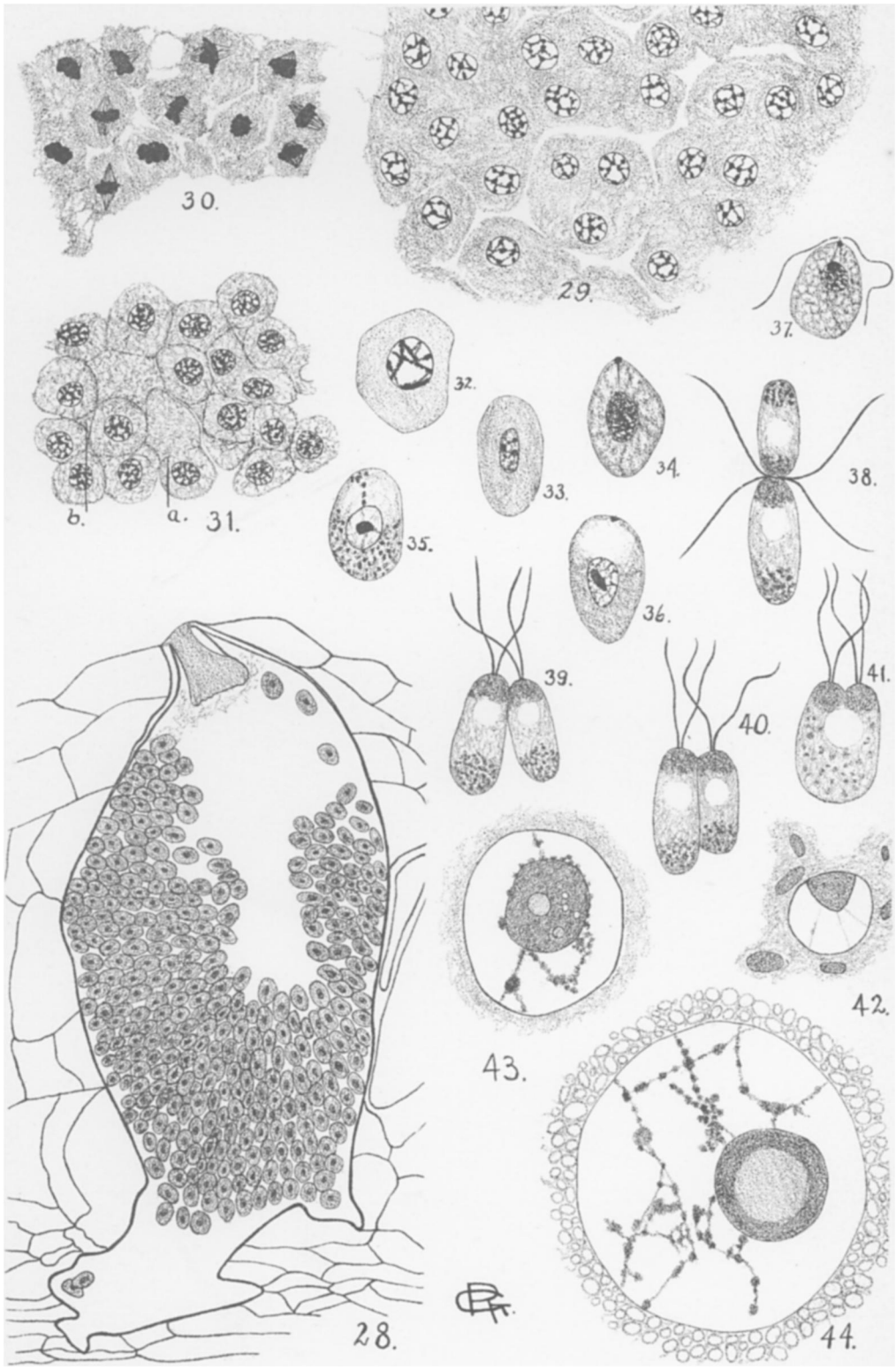

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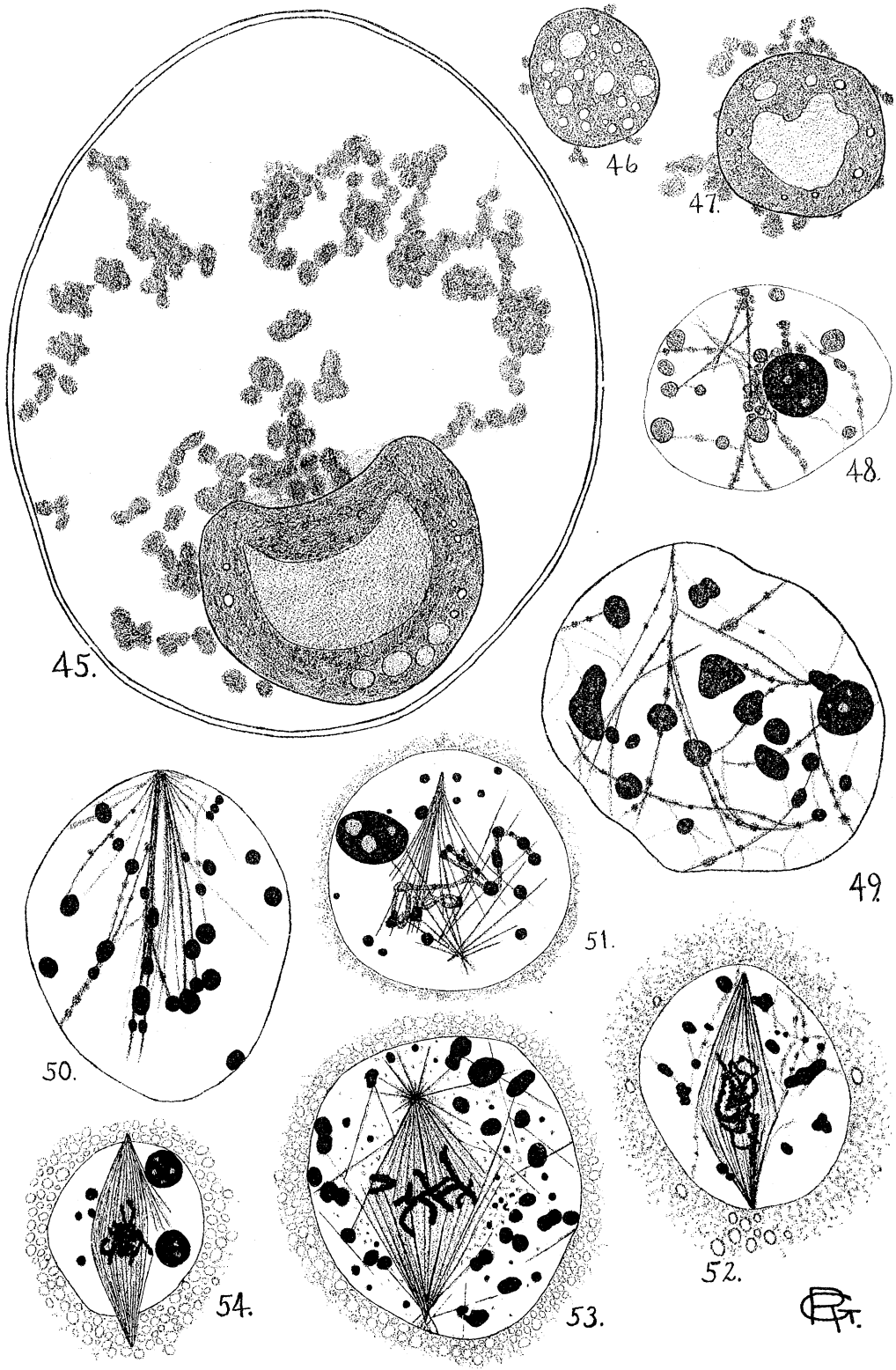

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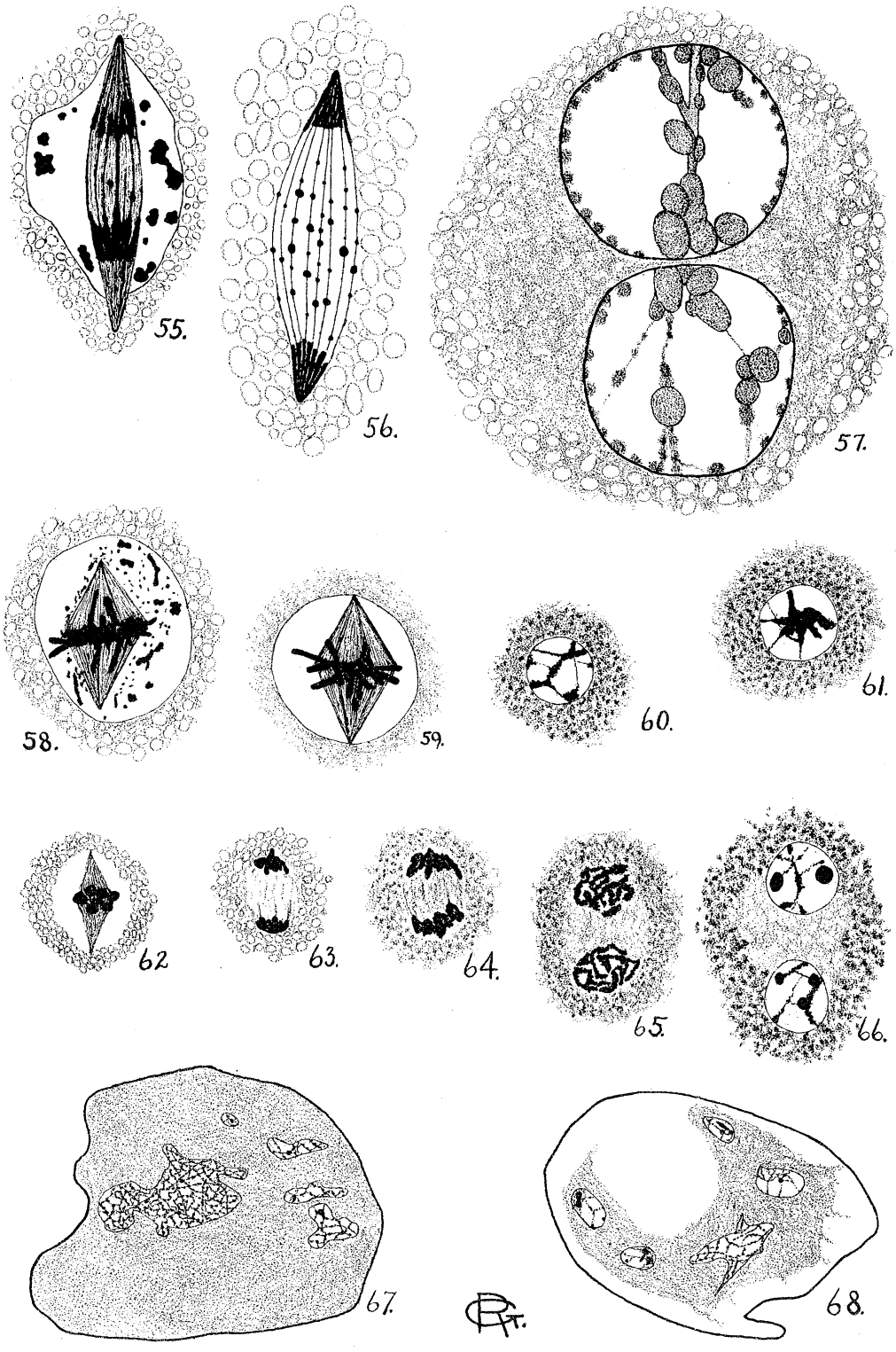
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FIG. 44.—A nucleus from a half-grown zoosporangium; nucleolus with a single central vacuole; connections between the chromatin spherules unusually well developed; ×2000.

FIG. 45.—Enlarged drawing of the nucleus of fig. 15; ×2000.

FIG. 46.—A nucleolus with many small vacuoles; ×2000.

FIG. 47.—A nucleolus in which several small vacuoles have coalesced into a single central vacuole; ×2000.

FIG. 48.—The beginning of prophase from a tetranucleate cyst in which the other three nuclei were well advanced in mitosis; ×2000.

FIG. 49.—Early prophase in the primary nucleus; ×2000.

FIG. 50.—Later prophase in the primary nucleus; no indication of the opposite pole could be found; ×2000.

FIG. 51.—Prophase in a binucleate cyst, showing formation of the second pole of the spindle and of the spirem; ×2000.

FIG. 52.—Late prophase with spirem entirely within the spindle; third division; ×2000.

FIG. 53.—Metaphase in primary nucleus, showing chromosomes, masses of residual chromatin, and irregular disposition of fibers through nuclear cavity; aster at one pole largely accidental; ×2000.

FIG. 54.—Metaphase; spindle beginning to elongate, but spirem not yet completely segmented into chromosomes; third division; ×2000.

FIG. 55.—Anaphase, showing elongation of spindle and residual chromatin; fourth division; ×2000.

FIG. 56.—Early telophase; probably fifth or sixth division; ×2000.

FIG. 57.—Late telophase, showing persistence of outline of primary nucleus; first division; ×2000.

FIG. 58.—Late prophase in an intermediate nucleus; residual chromatin partly finely divided; ×2000.

FIG. 59.—Metaphase in similar nucleus; no residual chromatin; ×2000.

FIGS. 60, 61.—Resting nucleus and prophase of second type of mitosis from the same cyst; ×2000.

FIG. 62.—Metaphase, second type of mitosis; ×2000.

FIGS. 63–66.—Telophases, second type of mitosis; ×2000.

FIGS. 67, 68.—Cysts with irregular nuclei which are interpreted as the products of amitosis; ×334.